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P 25,452

ABC

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Anhydrol Forte — containing 20% w/v Aluminium Chloride Hexahydrate — is supplied in a specially designed roll-on bottle. Patients with hyperhidrosis can therefore benefit from the proven effectiveness of the active ingredient, whilst minimising the occurrence of irritation.

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Prescribing Information

Presentation
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Indication
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Directions for use
Apply to the affected sites at night as required and allow to dry. Wash off in the morning.

Precautions
Restrict application to affected site only. Keep away from eyes.

Package quantity
10ml glass roll-on bottle.

PL 0173/0030 PA 278/7/1

Further information is available from:
Dermal Laboratories Limited,
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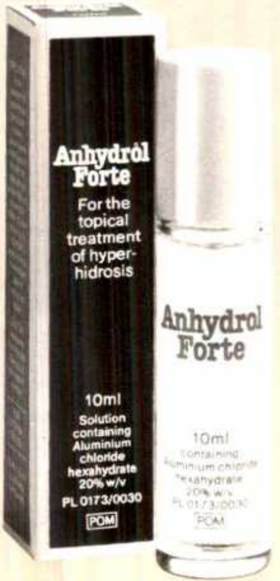
Anhydrol is a registered trade mark.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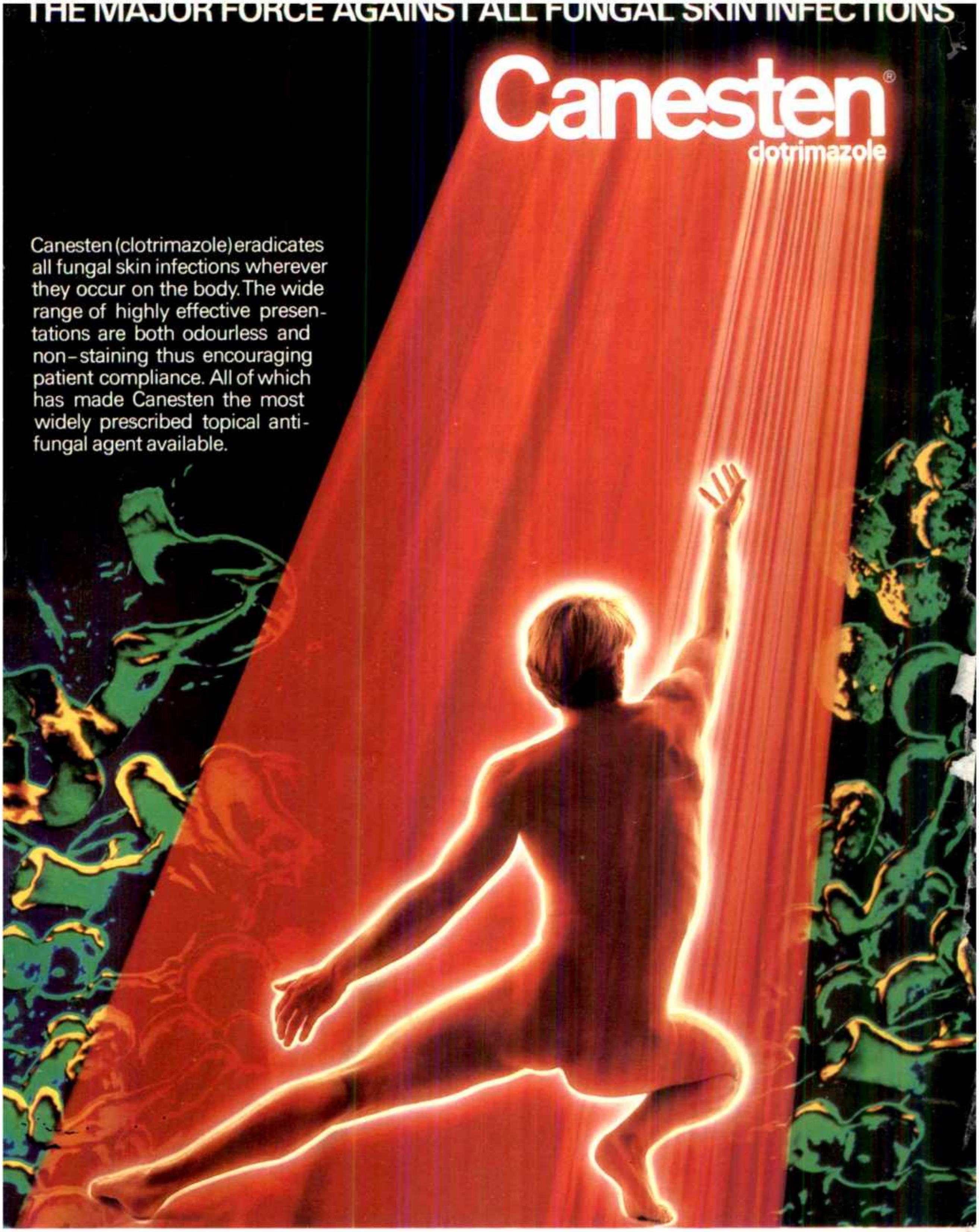

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Canesten (clotrimazole) eradicates all fungal skin infections wherever they occur on the body. The wide range of highly effective presentations are both odourless and non-staining thus encouraging patient compliance. All of which has made Canesten the most widely prescribed topical anti-fungal agent available.

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mazole in 30% isopropanol. *Solution* A clear solution containing 1% clotrimazole in polyethylene glycol. *Powder* A white powder containing 1.0%
mazole. **Indications** All fungal skin infections due to dermatophytes, yeasts moulds and other fungi including ringworm (tinea) infections. **Dosage**
Administration *Cream, Atomiser Spray, Solution* Apply to the affected area 2 – 3 times daily. *Powder* as above and may also be dusted inside articles
thing and footwear which are in contact with the infected area as a prophylactic against re-infection. **Side-effects** Rarely patients may briefly
rience a transient local mild burning or irritation. **Precautions** Canesten atomiser spray should not be used near naked flame; should not be allowed
me into contact with the eyes, ears or mucous membranes, and should not be inhaled. **Basic NHS costs** *Cream 20g* £1.67, *50g* £3.91. *Atomiser*
40ml £4.49. *Solution 20ml* £2.09. *Powder 30g* £1.21. **Product Licence Numbers** *Cream* PL 0010/0016 *Atomiser Spray* PL 0010/0060 *Solution*
10/0082 *Powder* PL 0010/0067.

Further information is available from

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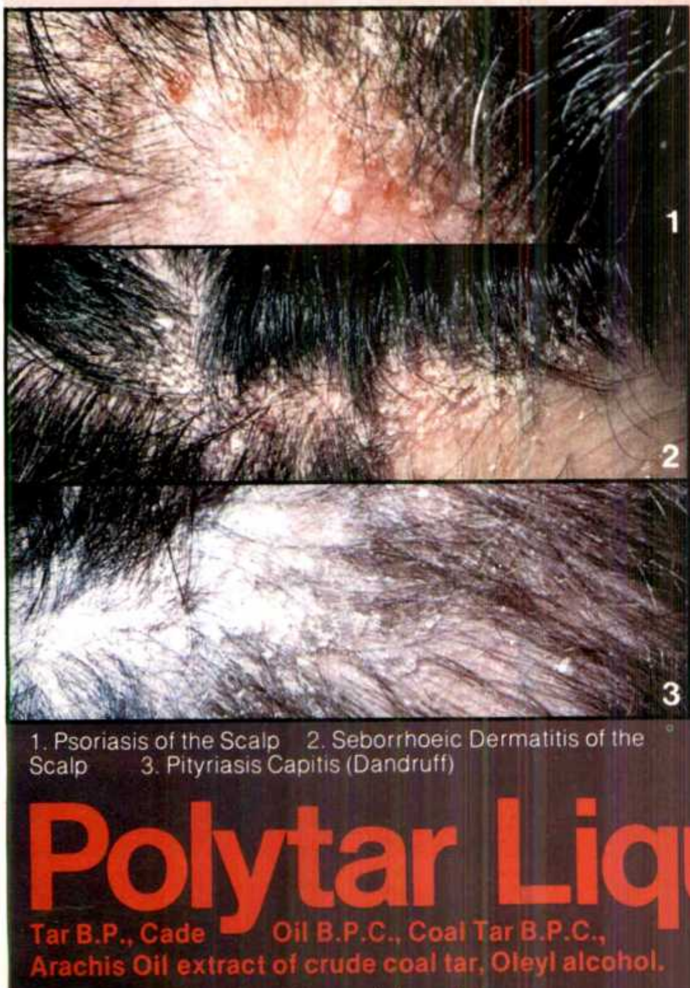
Common Disorders of the Scalp.



1. Psoriasis of the Scalp 2. Seborrhoeic Dermatitis of the Scalp 3. Pityriasis Capitis (Dandruff)

They all have one thing in common — the treatment

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Tar B.P., Cade Oil B.P.C., Coal Tar B.P.C., Arachis Oil extract of crude coal tar, Oleyl alcohol.

Acknowledged as ideal for long-term control.

NEW Polytar Plus with protein

Prescribing Information.

Presentation Polytar Liquid is a concentrated, antiseptic tar-medicated scalp cleanser containing the following active ingredients:

Tar B.P.	0.3%
Cade Oil B.P.C.	0.3%
Coal Tar B.P.C.	0.1%
Arachis Oil extract of crude coal tar	0.3%
Oleyl alcohol	1.00%

Polytar Plus contains 3.0% hydrolysed animal protein, in addition to the above.

Uses Polytar Liquid and Polytar Plus are indicated in the treatment of scalp disorders such as psoriasis, seborrhoea, dandruff, eczema and pruritus. They are also of value in the removal of pastes and pomades used in the treatment of psoriasis.

Dosage and Administration The hair should be wetted and sufficient applied to produce an abundant lather. The scalp and adjacent areas should be vigorously massaged with the fingertips. The hair should then be thoroughly rinsed and the procedure repeated.
Polytar Liquid or Polytar Plus should be used once or twice weekly.

Contra-indications, warnings, etc. Nil

Pharmaceutical Precautions Store in a cool place

Packs and Prices

	Pack	Basic NHS price
Polytar Liquid	65ml	57p
	150ml	82p
	350ml	£1.48
	1 litre	£3.90
Polytar Plus	150ml	90p

Product Licence Number
Polytar Liquid PL0174/5016 Polytar Plus PL0174/0037

Full information and Data sheet on request.

Stiefel Laboratories (UK) Limited
Wellcroft Road, Slough SL1 4AQ

Skin

P.F. MILLINGTON and R. WILKINSON

An up-to-date review of the fundamentals of the biology and mechanics of human skin, this book has as its major theme the interaction and dependence of the integrity of skin on cell turnover, nutrition, control mechanisms and disease. The structure and function of the dermis, including vasculature and nervous control, the extracellular fibres, collagen and elastin are reviewed, attention being drawn to the variations in quality, texture and behaviour of different regions of the body. Other topics include ageing, lines, markings and the use of cosmetic treatments.

£35.00 net

Biological Structure and Function 9

CAMBRIDGE UNIVERSITY PRESS

KING FAISAL UNIVERSITY, DAMMAM, SAUDI ARABIA

Academic Dermatologists

Faculty positions are available in the Department of Dermatology, College of Medicine and Medical Sciences, King Faisal University, Dammam, Saudi Arabia.

The Department of Dermatology is structured on the North American pattern. It has four faculty positions of which only two are currently filled. The department is undergoing rapid expansion and has ambitious plans for teaching and research. Teaching programmes for undergraduates and interns are already established and a residency programme is expected to commence in 1983. Current research activities are in epidemiology of skin diseases, leishmaniasis and mycology. Clinical activities are based at the Dermatology Clinic of the new 380 bed Teaching Hospital which serves as a referral centre for the Eastern province of Saudi Arabia.

These positions offer a unique opportunity to participate in the establishment of a new teaching department, practice tropical dermatology, interact with professionals from many countries and to explore another geographical area and culture.

Applications are invited from qualified Dermatologists with a background in academic dermatology. Appointments will be made at levels commensurate with experience and current positions.

Salaries are highly competitive and negotiable. Contracts are for one year and renewable. Instruction is in English. Benefits include furnished housing, air tickets to and from Saudi Arabia once per year for a family of four, 60 days paid annual leave, monthly transport allowance, generous luggage overweight allowance and educational allowance for children. No Saudi Income Tax.

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College of Medicine and Medical Sciences
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When you need to be strong.

❛ In chronic eczema, for example in the atopic variety, it may well be better to use strong topical steroids for short courses, e.g. two weeks, to try to clear the eczema and then stop treatment, rather than to use weaker steroids on an indefinite basis. ❜

Fry L. Update: Mar 1978 681-6

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it works on even long-standing, stubborn difficult dermatoses.

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it works quickly, so minimising the risk of steroid side effects.

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e effects

h all topical corticosteroids local atrophic changes may ssibly occur following prolonged and intensive treatment. o, prolonged use of large amounts or treatment of ensive areas may produce the features of hypercorticism. s is more likely to occur in infants and children, and with clusion. In infants, the napkin may act as an occlusive essing.

ovided the weekly dosage of Dermovate is less than 50 ams in adults, any pituitary-adrenal suppression is likely to be ansient with a rapid return to normal values once the short urse of steroid therapy has ceased. The same applies to ren given proportionate dosage.

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Rarely, treatment of psoriasis with corticosteroids (or its withdrawal) is thought to have provoked the pustular form of the disease.

Precautions

Long-term continuous therapy should be avoided, particularly in infants and children in whom adrenal suppression can occur even without occlusion. The face, more than other areas of the body, may exhibit atrophic changes after prolonged treatment with potent topical corticosteroids.

Appropriate chemotherapy should be used whenever infection of the skin is present. Any spread of infection requires withdrawal of topical corticosteroid therapy. In general, topical steroids should not be used extensively in pregnancy, i.e., in large amounts or for prolonged periods.

Contra-indications

Rosacea, acne and peri-oral dermatitis. Bacterial, fungal or viral diseases of the skin. Hypersensitivity to the preparation.

Basic NHS cost

(exclusive of VAT)

Dermovate Cream or Ointment 25 gram tubes £2.07 (also available in 100 gram tubes).

Presentation	**Product licence number**
Dermovate Cream:	4/0219
Dermovate Ointment:	4/0220

Glaxo Leaders in topical steroid therapy

Further information on Dermovate is available from:
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Minocin
minocycline hydrochloride
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Dosage Adults and children over 12 years. One tablet twice daily. Minocin may be taken with meals. Treatment of acne should be continued for a minimum of six weeks. **Contra-indications** Known tetracycline hypersensitivity. Systemic lupus erythematosus. **Precautions** Absorption is impaired by iron and antacids containing calcium, magnesium and aluminium salts. **Warnings and side effects** Gastro-intestinal disturbances are the side effects most commonly reported. A yellow to brown discoloration of teeth is a possible sequela of treatment during pregnancy or early childhood (up to 8 years of age). **Presentation** Beige tablets containing 50mg of minocycline as the hydrochloride, supplied in bottles of 100 at a basic NHS cost of £20.23. PL/0095/0062. MINOCIN is a trademark. Full prescribing information is available on request.

Lederle **Lederle Laboratories** Division of Cyanamid of Great Britain Ltd, Fareham Road, Gosport, Hants PO13 0AS (Tel: 0329-236131)

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so easy to use

Betnovate

betamethasone valerate

Scalp Application

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The preparation has an immediate cooling effect and gives rapid relief from the itching and inflammation associated with seborrhoeic dermatitis and other troublesome scalp conditions.

Betnovate Scalp Application is colourless, odourless and does not stain or leave unsightly residue in the hair.

Specially formulated; specially packed to make treatment easy

Prescribing information

Dosage and administration
Apply sparingly night and morning until improvement occurs. With Betnovate Scalp Application, improvement may be sustained by applying once a day or less frequently.

Contra-indications
Viral and dermatophyte infections of the scalp. Hypersensitivity to the preparation.

Precautions
Care must be taken to keep the preparation away from the eyes. Do not use near a naked flame. In infants and children avoid long term continuous topical therapy where possible, as adrenal suppression can occur even without occlusion. In general, topical steroids should not be used extensively in pregnancy, i.e., in large amounts or for prolonged periods.

Side effects
Betnovate Scalp Application is usually well tolerated, but if ns of hypersensitivity appear, lication should be stopped diately. With all topical costeroids, when extensive areas are treated, sufficient systemic absorption may occur to produce the features of hypercorticism. This is more likely to occur in infants and children with occlusion, or when treatment is prolonged. Local atrophy may occur after prolonged treatment under occlusion. Rarely, treatment of psoriasis with corticosteroids (or its withdrawal) is thought to have provoked the pustular form of the disease.

Basic NHS cost (exclusive of VAT)
30ml – £1·70

Product Licence number
4/5133

Further information on Betnovate (trade mark) Scalp Application is available from:
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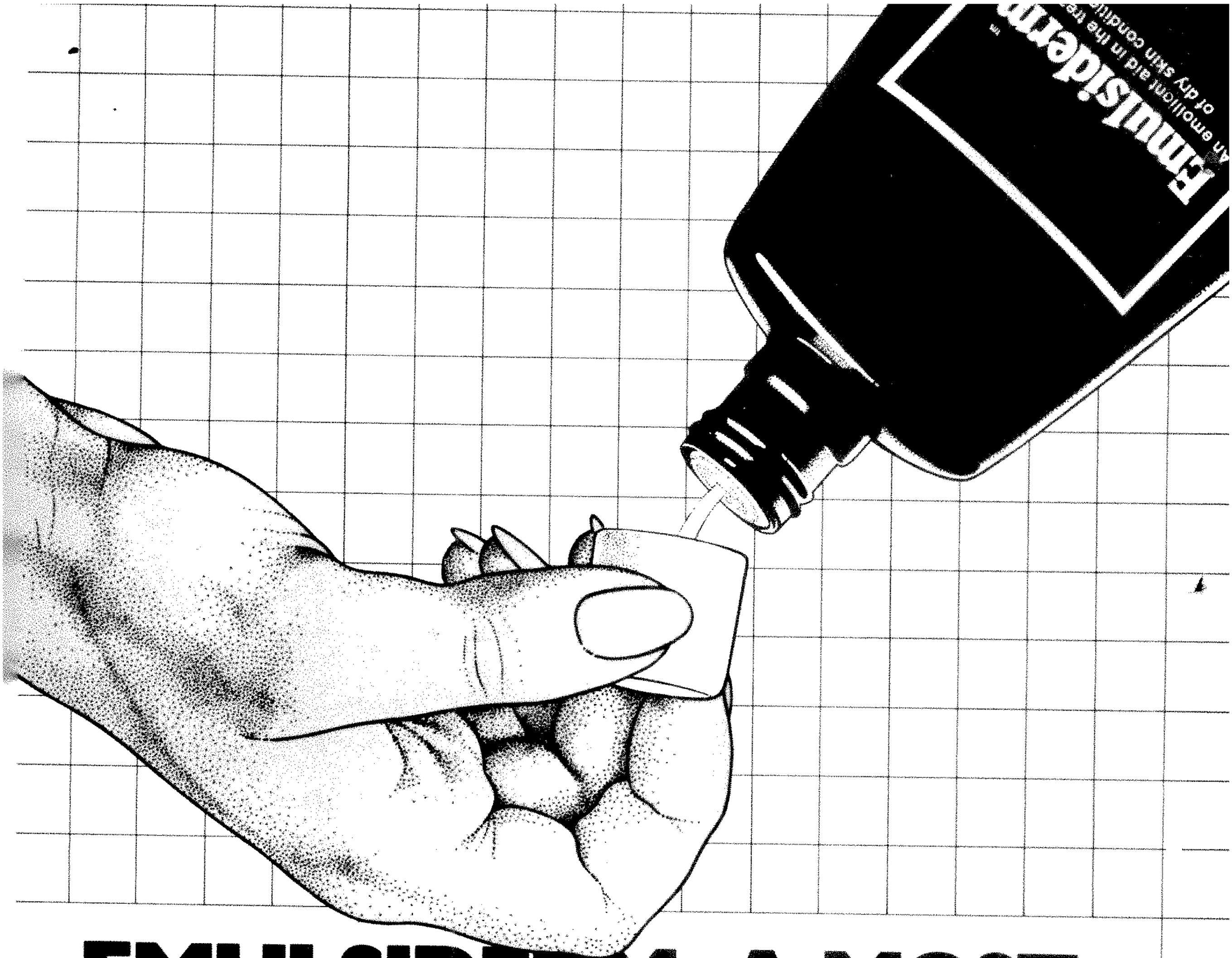
Leaders in topical steroid therapy



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Used in the bath, Emulsiderm disperses rapidly and thoroughly — ensuring effective coverage of the patient's skin.

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Presentation: Pale blue/green emulsion containing Benzalkonium Chloride BP 0.5%, Liquid Paraffin BP 25.0%, Isopropyl Myristate BPC 25.0%.
Uses: An aid in the treatment of dry skin conditions, especially those associated with eczema, ichthyosis or xeroderma.
Directions: Always shake bottle before use. In the bath: Add 2-3 capfuls to a 6-8 inch bath of warm water. For infant bathing use 1-2 capfuls. Soak for 5-10 minutes. Pat dry. On the skin: Rub a small amount of undiluted emollient into the dry areas of skin until absorbed.
Contra-indications, warnings etc:
Keep away from eyes. Take care to avoid slipping in the bath.
Package quantity: 250 ml polythene bottles with a 10 ml measuring cap.
PL 0173/0036. PA 278/8/1.
Further information is available from:
Dermal Laboratories Limited,
Gosmore, Hitchin, Herts. SG4 7QR
Emulsiderm is a registered trademark

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Altered polymorphonuclear leukocyte responses in psoriasis: chemotaxis and degranulation

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SUMMARY

Chemotactic activities of circulating polymorphonuclear leukocytes (PMN) were determined in twenty patients with psoriasis and twenty healthy control persons. After serial dilution of the complement split product C5a and the formylated tripeptide f-met-leu-phe (FMLP), chemotaxis profiles showed that PMN migration toward both chemotaxins was significantly increased in psoriasis. In addition, PMN from psoriatic patients responded to chemotaxins at much lower concentrations compared with controls.

The liberation of (lysosomal) β-glucuronidase was also determined in cytochalasin B-treated cells confronted with increased concentrations of the chemotaxins. Secretion of this marker enzyme started at lower concentrations in PMN derived from psoriatic patients.

Our observations demonstrate migratory and secretory hyper-responsiveness of PMN from psoriatic patients. This may play a role in perpetuating the psoriatic tissue reaction.

In recent years several abnormalities of PMN function in psoriasis have been observed, including altered chemotactic activities against various chemotaxins (Wahba *et al.*, 1978; Tagami & Ofuji, 1976; Langner *et al.*, 1978; Michaelsson, 1980; Kawohl *et al.*, 1980). On the basis of these findings it was suggested that abnormally functioning PMN contribute to the psoriatic tissue reaction (Kawohl *et al.*, 1980). However, in a more recent study (Breathnach, Carrington & Black, 1981) no evidence was found to support this.

In the present study chemotactic profiles of PMN from patients with psoriasis as well as the degranulation capacity of PMN after specific stimulation are reported. The results support the concept of hyper-responsive PMN being present in patients with psoriasis.

METHODS

All patients were in hospital for treatment of psoriasis (eleven males and nine females, 25–83

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TABLE 1. Clinical data and maximum chemotactic activities of twenty patients with psoriasis

No.	Age/Sex	Psoriasis duration (years)	Time since relapse (months)	Type of psoriasis	Max. chemotactic activity (Cells/HPF) C5a ($1:10^{-2}$)	FMLP (1×10^{-9})
1	25 M	8	6	guttate	209	102
2	29 M	10	4	CS	218	97
3	31 F	20	5	CS	203	103
4	31 M	14	2	pustular	226	112
5	32 F	9	1·5	CS	199	97
6	38 F	10	4	guttate	229	101
7	39 F	2	6	CS	199	98
8	39 M	12	1·5	CS	208	94
9	40 M	2	1	erythr.	209	105
10	45 M	3	4	CS + arthr.	186	91
11	46 M	20	2	guttate	227	98
12	50 F	31	3	CS	194	93
13	50 M	2	5	CS	226	94
14	52 F	26	6	CS	230	100
15	60 M	23	2	erythr.	223	106
16	61 F	31	2	CS + arthr.	212	101
17	70 F	5	3	CS	224	96
18	72 F	10	0·5	CS	207	95
19	74 M	30	2	erythr.	214	108
20	83 M	20	6	CS	220	94

CS = chronic stationary type; erythr. = psoriatic erythroderma; arthr. = psoriatic arthropathy.

years old, Table 1). They showed more than 30% of skin affected by psoriatic lesions. The control group consisted of healthy students and medical personnel (thirteen males and seven females, 21–42 years old).

Preparation of PMN

Blood samples were taken before any therapy was started. PMN were isolated as previously described (Kawohl *et al.*, 1980) using a modification of the method of Henson (1971). Briefly, venous blood was drawn into 1/6 volume of acid citrate dextrose, centrifuged and the supernatant removed. The sediment was mixed with 2·5% gelatin in 0·9% NaCl and the cell suspension was allowed to sediment at 37°C for 30 min.

The PMN-containing supernatant was centrifuged and contaminating erythrocytes were lysed with 0·85% NH_4Cl in distilled water. The PMN suspension was centrifuged and washed with medium TC 199 (Seromed, Munich). The final cell preparation contained more than 90% neutrophils with a viability of greater than 95% as assessed by trypan blue exclusion.

Chemo-attractants

Fresh human serum was activated in the presence of 1 M ε-aminocaproic acid (EACA, Serva, Heidelberg) with zymosan (5 mg/ml at 18°C for 30 min and 37°C for 60 min Sigma, Munich). The chemotactic C5a was obtained by passage of activated serum which was titrated with cold

1 M HCl to pH 4 over a Sephadex G75 column (Pharmacia, Uppsala) with 0·1 M acetate buffer, pH 4·6 as eluant. The post-albumin fractions (approximate mol. wt range 5000–30,000), which were chemotactic for PMN, were concentrated by ultrafiltration (Immersible-CX, NMWL 10,000 daltons, Millipore Bedford, Ma). The concentrated C5a fractions were adjusted to pH3 with 0·01 N HCl, then portioned in small volumes and stored below −70°C until use.

For chemotaxis C5a was diluted just before use with TC 199, containing 0·1% human serum albumin (HSA; Behringwerke AG, Marburg).

N-formyl-L-methionyl-L-leucyl-L-phenylalanine (FMLP, Serva, Heidelberg) was dissolved in DMSO (10^{-2} M). FMLP was diluted to 10^{-5} M in TC 199 containing 0·1% HSA, portioned at this concentration and stored below −70°C until use.

The concentration of DMSO never exceeded 0·1% and did not affect chemotaxis and enzyme release at this concentration (data not shown).

Boyden chamber chemotaxis

Chemo-attractants were placed in the lower compartment (100 μl) of a modified Boyden chamber of the blind well type. The compartments of the chamber were separated by a membrane filter (cellulose nitrate filter, pore size 5·0 μm, Sartorius GmbH, Göttingen). The upper compartments were filled with 100 μl of the PMN suspension (2×10^6 cells/ml, 1% HSA in TC 199). The chambers were incubated for 2 h at 37°C. Thereafter the filters were fixed, stained and counted as previously described (Kawohl *et al.*, 1980).

The chemotactic activity (CA) was expressed as the number of cells which migrated to the bottom side of the filter (cells/HPF) (Kawohl *et al.*, 1980). Each experiment was carried out in duplicate. Medium TC 199 containing 0·1% HSA was used as a negative control.

Enzyme determinations

β-glucuronidase was determined by the method of Brittinger *et al.* (1968). As medium for the stimuli (C5a and FMLP) phosphate-buffered saline (PBS, Dulbecco) was used. For each experiment, which was done in duplicate, 3×10^6 PMN in 100 μl colourless medium TC 199 (Seromed, Munich) were used. The cells were first incubated 10 min at 37°C with cytochalasin B (5 μg/ml; Serva, Heidelberg). Then 200 μl stimulus was added and the mixture was incubated for 60 min at 37°C.

Thereafter the cells were centrifuged (800 ***g***, 10 min) and 200 μl of the β-glucuronidase-containing supernatant was incubated with 300 μl 0·1 M sodium acetate buffer (pH 4·6), 50 μl phenolphthalein-β-D-glucuronide (0·01 M in 0·9% NaCl, pH 7·0; Serva, Heidelberg) for 18 h at 37°C and then 1·0 ml 0·2 M glycine buffer pH 10·5 was added. The extinction was determined at 546 nm.

For evaluation of the total enzyme content, 3×10^6 PMN in 100 μl colorless TC 199 and 200 μl PBS were frozen and thawed three times and after centrifugation 200 μl supernatant were assayed for β-glucuronidase as described above. β-glucuronidase activity was expressed as per cent of total. In each experiment the cytoplasmatic enzyme LDH was determined (LDH-test, Boehringer, Mannheim). LDH never exceeded 4% of total enzyme content.

RESULTS

The clinical data and maximum PMN migratory responses of the psoriatic patients are shown in Table 1. Various types of psoriasis with various lengths of history were included. No correlation between chemotactic activity (CA), age, sex, type and duration of the disease could be seen.

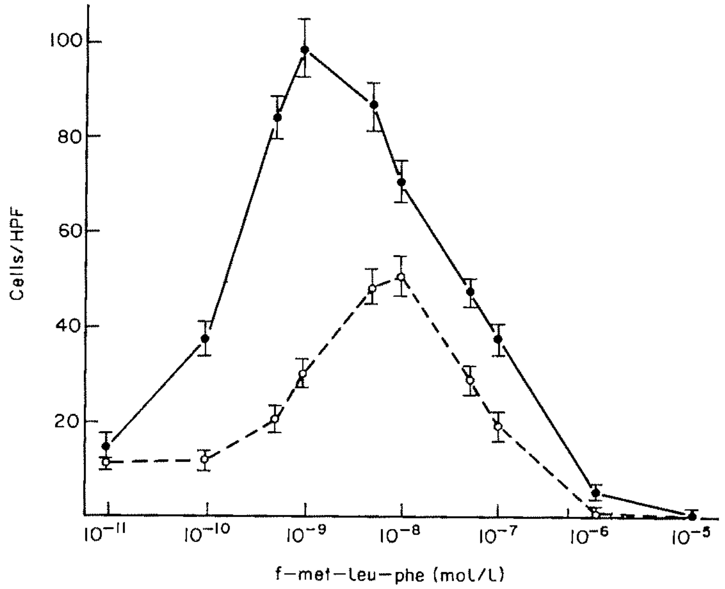

FIGURE 1. Chemotactic profiles of PMN from patients with psoriasis (—●—) compared with healthy controls (- -○- -). The concentrations of f-met-leu-phe as a chemo-attractant range from 10^{-11} to 10^{-5} mol/l. Chemotactic activity is expressed as cells/HPF counted on the undersurface of the chemotactic filters.

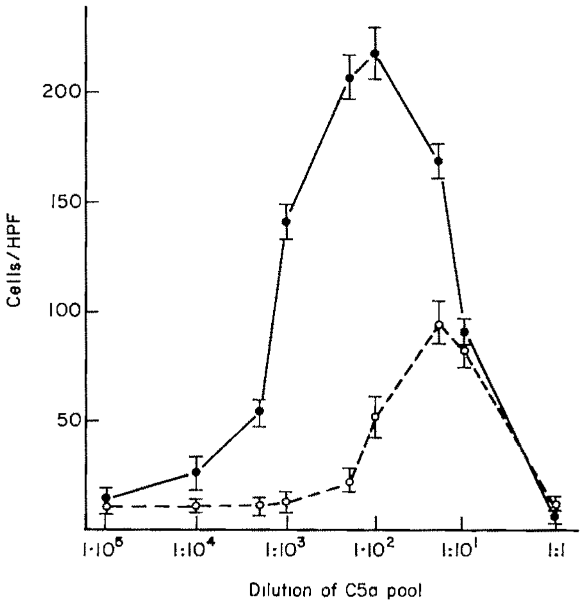

FIGURE 2. Chemotactic profiles of PMN from patients with psoriasis (—●—) compared with healthy controls (- -○- -) using various dilutions of human C5a pool. As with f-met-leu-phe greatly increased chemotactic profiles in psoriatic patients can be seen.

When the chemotaxins were serially diluted and CA was subsequently determined, biphasic responses were seen resulting in nearly bell-shaped curves (Figs 1 & 2). The number of PMN migrating through the filters showed a steep rise as the chemotaxin concentrations increased. With further increase in strength of the chemotaxins, the number of responsive PMN became successively smaller. Maximum chemotactic activities were obtained with a 1 : 100 dilution of the C5a stock solution or 10^{-9} mol/l of FMLP (Table 1). By this method chemotactic profiles were obtained for both chemotaxins (Figs 1 & 2).

Psoriatic as well as the control PMN appeared to respond similarly, showing increasing and decreasing numbers of migrating cells as the concentration of the chemotaxins increased. However, there were marked quantitative differences between the two groups, PMN from psoriatic patients being much more sensitive (Figs 1 & 2). For FMLP, peak values of approximately 100 cells per HPF were seen at 10^{-9} M whereas in the control group this was noted at a ten times higher concentration (Fig. 1).

When C5a was used as chemotaxin, the difference between the chemotaxis profiles of the two groups was even more pronounced. At a 1 : 10 dilution of the C5a pool the chemotactic activity of psoriatic PMN was twice as high as for the control PMN (Fig. 2). With further dilution of the chemotaxin this difference was greater, demonstrating a high sensitivity of PMN in psoriatic patients.

Liberation of β-glucuronidase

Determination of β-glucuronidase as a marker enzyme for azurophilic granule discharge was carried out by exposing the cells to various concentrations of the chemotaxins in the presence of cytochalasin B. The results show that a considerable amount of the enzyme can be detected extracellularly (Table 2). Significant differences between the two groups are seen at chemotaxin concentrations of 10^{-6} to 10^{-8} mol/l FMLP, e.g. with 10^{-8} mol/l FMLP nearly twice as much β-glucuronidase was liberated in the patient group as compared with the controls (Table 2). The differences were less profound when C5a was used, although there were still significant differences detectable between the two groups (Table 3). This was the case for 1 : 10 and 1 : 100 dilutions of the C5a pool. Higher dilutions tended to show baseline values in both groups (Table 3).

TABLE 2. Liberation of β-glucuronidase from PMN in the presence of various concentrations of FMLP

Concentration of FMLP (mol/l)	Enzyme activity (% of total) Psoriasis (n = 10)	Healthy controls (n = 10)	Significance P
10^{-6}	21·7 ± 1·3	15·0 ± 1·2	0·001
10^{-7}	16·0 ± 1·1	10·2 ± 1·1	0·001
10^{-8}	11·5 ± 0·9	5·8 ± 0·8	0·001
10^{-9}	6·0 ± 0·7	4·0 ± 0·7	0·001
10^{-10}	4·9 ± 0·7	4·0 ± 0·8	NS
10^{-11}	4·1 ± 0·8	3·8 ± 0·7	NS
Control	3·9 ± 0·7	4·1 ± 0·9	

NS = Not significant.

TABLE 3. Liberation of β-glucuronidase from PMN in the presence of various concentrations of C5a

Dilution of C5a stock solution	Enzyme liberation (% of total) Psoriasis (n = 10)	Enzyme liberation (% of total) Healthy control (n = 10)	Significance P
1:10	11·7 ± 1·3	7·4 ± 0·7	0·001
1:100	7·3 ± 0·8	5·0 ± 0·6	0·001
1:1000	5·1 ± 1·1	4·4 ± 0·6	NS
1:10,000	4·5 ± 0·7	4·0 ± 0·5	NS
1:100,000	4·1 ± 0·8	3·9 ± 0·6	NS
Control	4·0 ± 0·8	3·8 ± 0·7	NS

NS = Not significant.

DISCUSSION

Functional abnormalities of PMN recently reported in psoriasis include altered adherence (Sedgwick, Bergstresser & Hurd, 1980), NBT reduction (Cotterill *et al.*, 1974), chemotaxis (Wahba *et al.*, 1978; Tagami & Ofuji, 1976; Langner *et al.*, 1978; Michaelsson, 1980; Kawohl *et al.*, 1980) and phagocytosis (Wahba *et al.*, 1978). On the other hand in a study on neutrophil migration in patients with psoriasis Breathnach *et al.* (1981) were unable to confirm the findings of other authors. Among the difficulties linked with the establishment of standardized chemotaxis assays is the use of different chemotaxins, e.g. casein (Langner *et al.*, 1978), *E. coli* filtrate (Michaelsson, 1980), *Shigella* lipopolysaccharide-activated serum (Wahba *et al.*, 1978) or autologous serum (Kawohl *et al.*, 1980). In addition, different chemotaxis systems have been employed so that some of the results may not be directly comparable.

In the present study we have used two potent chemotaxins, C5a and FMLP. Both chemotaxins have been extensively employed in the study of neutrophil function in recent years. C5a may be generated endogenously in complement-mediated inflammatory reactions. FMLP is the synthetic analogue of bacterial chemotactic factors and thus appears to be relevant for infectious disease states (Schiffman, Corcoran & Wahl, 1975). Both C5a and FMLP are able to induce a variety of neutrophil responses (Showell *et al.*, 1976; Goldstein *et al.*, 1975; Boxer *et al.*, 1979) and separate receptors for both chemotaxins have been identified (Chenoweth & Hugli, 1978; Goetzl & Kum Yoke Hoe, 1979). Using these chemotaxins we have measured chemotactic activities of PMN over a wide range of concentrations. Chemotactic profiles were established by which further information on the sensitivity of PMN exposed to various concentrations of a stimulus could be obtained. Such experiments have not been done before.

PMN derived from patients were more sensitive to lower chemotaxin concentrations compared with the controls. In fact chemotactic migration towards both chemotaxins started at concentrations where normal PMN were still non-responsive. Furthermore, at each of the chemotaxin concentrations more than twice as many cells were stimulated to migrate compared with the controls. These results confirm the chemotactic hyper-responsiveness of PMN previously observed in psoriasis (Wahba *et al.*, 1978; Tagami & Ofuji, 1976; Langner *et al.*, 1978; Michaelsson, 1980). Wahba *et al.* (1978) found a significantly enhanced neutrophil chemotaxis towards lipopolysaccharide-activated serum in psoriasis. Using the agarose method

Michaelsson (1980) showed that random as well as active migration was increased in patients with psoriasis. *E. coli* filtrate was used as chemotaxin. Langner *et al.* (1978) as well as Michaelsson (1980) noted that this increase of PMN migration was most marked in severe widespread psoriasis whereas it was less pronounced in moderate psoriasis of the chronic stationary type. Since all of the patients reported in the present study suffered from extensive skin involvement the distinctions mentioned above could not be made.

Studies on the liberation of enzymes have not been reported in PMN from psoriatic patients. Our results obtained after exposure of PMN to high concentrations of chemotaxins in the presence of cytochalasin B demonstrate increased enzyme liberation in psoriatic patients. Since the concentrations of (cytoplasmic) LDH remained close to 4% of the controls throughout the experiments, specific degranulation of the azurophilic PMN granules is ascertained. Both observations, namely increased migratory activity as well as earlier enzyme secretion in PMN from psoriatic patients, may have a bearing on the disease process. Indeed, with PMN function greatly stimulated at subthreshold concentrations of a chemotaxin, the abnormalities shown here could contribute to the perpetuation of the psoriatic tissue reaction. Comparison of our present findings with those obtained in patients with other diseases involving PMN will be necessary.

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Enhanced release of inflammatory mediators from lithium-stimulated neutrophils in psoriasis

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SUMMARY

We have used lithium salts to stimulate degranulation in order to assess neutrophil activity in psoriasis. Evidence is presented for significant enhancement of degranulation of β-glucuronidase (primary granules) and vitamin B_{12}-binding protein (secondary granules) from lithium-stimulated neutrophils in psoriatic whole blood.

Basal levels of granule markers showed no significant difference between normal and psoriatic neutrophils. On the other hand, enzymes associated with neutrophil function (myeloperoxidase and catalase) were found to be markedly increased in resting psoriatic neutrophils.

An important recent development has been the observation that psoriatic neutrophils are 'activated' in terms of their neutrophil function. Wahba *et al.* (1978, 1979) observed enhanced chemotactic and phagocytic properties of isolated psoriatic neutrophils, and Sedgwick, Bergstresser & Hurd (1979, 1980) demonstrated increased adherence of isolated psoriatic neutrophils to glass-wool and nylon fibres. Recently, there have been a number of reports concerning the clearing of psoriasis in patients undergoing haemodialysis and peritoneal dialysis (McEvoy & Kelly, 1976; Buselmeier *et al.*, 1979; Glinski *et al.*, 1979), and it is now believed that improvement was a result of removal of neutrophils rather than some psoriatogenic factor.

Lithium salts are commonly used in the treatment of bipolar affective psychosis. Lazarus & Gilgor (1979) drew attention to reports that lithium salts adversely affect the course of psoriasis by direct or indirect mechanisms. They may do so by affecting neutrophil function, thus establishing a relationship between psoriasis and neutrophil activity. Lithium salts can dramatically affect neutrophil activity in terms of granulopoiesis, turnover and migration (Rothstein *et al.*, 1978). We have recently demonstrated that lithium salts have the capacity to induce directly the release of inflammatory mediators from neutrophils in normal whole blood, in the absence of phagocytic stimulation (Bloomfield & Young, 1982). The objective of this

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investigation was to demonstrate that lithium salts are capable of causing enhanced degranulation from psoriatic neutrophils. In addition, the results would demonstrate *in vitro* the possible effects which lithium might have in exacerbating psoriasis *in vivo*, i.e. by causing release of inflammatory mediators from neutrophils which have been shown to be activated.

METHODS

Thirty-two patients with chronic plaque-type psoriasis of mild to moderate severity, who were not receiving intensive topical or systemic treatment, were selected for this investigation. Thirty-two normal subjects were selected from healthy laboratory personnel and students with no known personal or family history of skin or other inflammatory conditions.

Test for degranulation

The method has been described recently by Bloomfield & Young (1982). Whole blood was collected by venepuncture into 50 iu/ml sodium heparin. To two 5-ml aliquots were added either 20 μl lithium chloride (to stimulate degranulation) or sodium fluoride (to inhibit degranulation), so that the final concentrations were 10 mmol/l and 1 mmol/l respectively. Also added were 20 μl of magnesium chloride and glucose (final concentrations, 1 mmol/l). The samples were incubated at 37° for 30 min, and layered onto 3-ml Hypaque-Ficoll gradients for isolation of the neutrophils as described by Ferrante & Thong (1978). Recovery was found to be about 80% and viability greater than 98%. Triton X-100 was added (final concentration, 0·1%) to lyse the cells for enzyme assays. The amount of degranulation taking place was calculated from the *residual* activity in the lysed cells after treatment with lithium, expressed as a percentage of the control sample, containing fluoride.

Enzyme assays

Fresh isolated neutrophils, lysed with 0·1% Triton X-100, were employed for measurement of basal levels of enzymes and vitamin B_{12}-binding protein. Extracted cells in the degranulation test were assayed for the granule markers only. Assays for the markers for primary (β-glucuronidase) and secondary (vitamin B_{12}-binding protein) granules were described by Kane & Peters (1975). Myeloperoxidase was measured using the methods of Klebanoff (1965); catalase was assayed by the methods described by Baudhuin *et al.*, (1964) and proteins were measured by the Lowry technique (Lowry *et al.*, 1951).

TABLE 1. Degranulation of β-glucuronidase and vitamin B_{12}-binding protein from normal and psoriatic neutrophils

	Percentage degranulation*				
	Normal neutrophils		Psoriatic neutrophils		
β-glucuronidase	15·0 ± 7·10	(32)	34·0 ± 13·0	(32)	$P < 0·001$
Vitamin B_{12}-binding protein	19·4 ± 7·80	(32)	38·5 ± 12·5	(32)	$P < 0·001$

*Percentage degranulation is the amount of enzyme or protein released in 30 min at 37°C, expressed as a percentage of the initial level. Values given are mean ± s.d. Number of subjects are in parentheses.

TABLE 2. Basal levels of enzymes and vitamin B_{12}-binding protein in normal and psoriatic neutrophils

	Specific activity*				
	Normal neutrophils		Psoriatic neutrophils		
β-glucuronidase	1·04 ± 0·20	(30)	1·30 ± 0·36	(30)	N.S.
Vitamin B_{12}-binding protein	1120 ± 350	(30)	1150 ± 398	(30)	N.S.
Myeloperoxidase	0·93 ± 0·34	(20)	4·14 ± 3·10	(20)	$P < 0·001$
Catalase	7·42 ± 3·35	(10)	49·3 ± 26·3	(10)	$P < 0·001$

*Specific activity: mUnits/mg (β-glucuronidase and catalase); pg/mg (vitamin B_{12}-binding protein); ΔOD/min/mg (myeloperoxidase). Values given are mean ± s.d. Numbers of determinations are in parentheses.

RESULTS

Degranulation was induced by lithium from normal and psoriatic neutrophils. Table 1 shows the percentage degranulation of primary and secondary granule content from normal and psoriatic neutrophils, and demonstrates a considerable enhancement of degranulation from psoriatic cells ($P < 0·001$). Therefore, psoriatic neutrophils have a greater capacity to release their inflammatory mediators (*in vitro*). There was no significant correlation between primary (β-glucuronidase) and secondary (vitamin B_{12}-binding protein) degranulation in either normal ($r = 0·34$) or psoriatic subjects ($r = -0·31$).

Basal levels of enzymes associated with neutrophil function were measured in isolated normal and psoriatic neutrophils, in addition to the markers for the primary and secondary granules (Table 2). There was no significant difference between the basal levels of the markers for primary and secondary granules in normal and psoriatic neutrophils. In contrast, myeloperoxidase and catalase showed significant increases compared with normal levels.

DISCUSSION

A number of studies concerning neutrophils and the pathogenesis of psoriasis have been reported, but this is the first major study demonstrating that psoriatic neutrophils have an increased capacity to release inflammatory mediators (e.g. lysosomal enzymes) compared with normal cells (Table 1). We have demonstrated that the percentage degranulation is significantly higher in psoriatic neutrophils from both primary and secondary granules compared with normal ($P < 0·001$). However, there was no statistical correlation between degranulation of primary and secondary granules in either group. This shows further evidence of the different response to stimuli by the two granule populations (Bainton, 1973). Since there was no significant difference between basal levels of the markers for primary and secondary granules between normal and psoriatic neutrophils (Table 2), it can be assumed that the increase in degranulation from psoriatic neutrophils could not be attributed to possible leakage due to increased basal enzyme levels.

The enzymes myeloperoxidase and catalase, which are associated with neutrophil function, showed a significant increase in psoriatic neutrophils compared with normal cells (Table 2). Halogenated compounds are produced in the presence of myeloperoxidase and these are bactericidal. It was proposed by Wahba *et al.* (1979) that the lower levels of surface bacteria on psoriatic skin were due to active neutrophils. We would like to extend this hypothesis to the increased production of bactericidal compounds, due to the increased levels of myeloperoxidase in psoriatic neutrophils. Catalase, another enzyme which detoxifies hydrogen peroxide, was also increased in psoriatic neutrophils, and adds further evidence for enhanced neutrophil activity in psoriasis.

There are a number of reports of exacerbation of psoriasis by lithium during treatment of bipolar affective psychosis (Skoven & Thormann, 1979). In a recent article, Heng (1982) observed that acneform eruptions were associated with high serum lithium levels (1·5–2·5 mEq/l) in several cases, and suggested that development of pustular episodes of psoriasis may be related to lysosomal enzyme release. We have observed significant degranulation in normal and psoriatic subjects using 5 mmol/l lithium chloride (data not shown) but chose 10 mmol/l as our working concentration having shown that it was not toxic at this concentration (Bloomfield & Young, 1982) and could stimulate significant degranulation in 30 min. In this communication we have demonstrated that psoriatic neutrophils have a greater capacity to degranulate in response to lithium compared with normal cells. We would therefore suggest that the exacerbation of psoriasis during lithium therapy might be due to enhanced release of inflammatory mediators by activated neutrophils.

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Molecular size of secreted and cell-associated plasminogen activators from cultured epidermal cells

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SUMMARY

The molecular sizes of secreted and cell-associated plasminogen activators from four cultured cell types were determined using an SDS-PAGE technique in which plasminogen and casein were included during polymerization of the polyacrylamide gel. The major bands of plasminogen activators secreted by human neonatal epidermal cells, human adult epidermal cells and transformed human squamous cells migrated the same distance as the high molecular weight band of authentic urokinase, indicating that the apparent molecular weight of these plasminogen activators was approximately 55,000 daltons. Plasminogen activator extracted from normal adult human epidermis also migrated with this major band of plasminogen activator, and a minor higher molecular weight band was also detected. In contrast, plasminogen activators secreted by transformed mouse squamous cells migrated between the high molecular weight band (~55K) and the low molecular weight band of urokinase (~32K), indicating that plasminogen activators of mouse epidermal cells differ from those of human epidermal cells. The mobility of the major bands of plasminogen activators detected in cell lysates of the four cell types was identical to that of secreted plasminogen activators.

Plasminogen activators (PA) are serine proteinases which catalyse the conversion of plasminogen to plasmin. These important fibrinolytic enzymes have been implicated in localized extracellular proteolysis by malignant cells (Christman, Silverstein & Acs, 1977; Reich, 1978; Quigley, 1979), activated macrophages (Unkeless, Gordon & Reich, 1974; Gordon, Unkeless & Cohn, 1974) polymorphonuclear leukocytes (Granelli-Piperno, Vassalli & Reich, 1977), trophoblast (Strickland, Reich & Sherman, 1976) and granulosa cells (Beers, Strickland & Reich, 1975; Strickland & Beers, 1976).

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Our laboratory has demonstrated increases in plasminogen activator activity in psoriatic epidermis which correlate with disease activity (Fraki, Briggaman & Lazarus, 1982; Fraki *et al.*, 1982). Epidermal cell cultures are pótent tools to investigate the role of plasminogen activator in epidermal physiology. We have shown that PA activity is enhanced by incubating epidermal cells with pemphigus IgG (Hashimoto *et al.*, 1982a). The present study characterizes the plasminogen activator in four types of cultured cells detected by a modified SDS-PAGE technique in which plasminogen and casein are included during polymerization of the polyacrylamide gel.

METHODS

Cell culture

Human epidermal cells from neonate foreskins and normal adult breast skin were cultured on 3T3 fibroblast feeder layers using a modification of the Rheinwald & Green (1975) technique (Hashimoto, Singer & Lazarus, 1982b). Human epidermal cells were grown in Dulbecco's Modified Eagle's Medium (DME) (B and B Research Labs, Baltimore, MD), supplemented with fetal calf serum (Gibco) to a final concentration of 20%, 20 ng/ml epidermal growth factor (Collaborative Research, Waltham, MA), 0·4 μg/ml hydrocortisone (Calbiochem–Behring, La Jolla, CA), 10^{-10} M cholera toxin (Schwartz Mann, Orangeburg, NY), 100 units/ml penicillin and 100 mg/ml streptomycin (Gibco). ME180 is a transformed human squamous cell line originating from a cervical carcinoma (Sykes *et al.*, 1970). PAM is a spontaneously transformed mouse squamous cell line originating from a normal mouse epidermal cell culture and was the generous gift of Dr Stuart Yuspa, National Cancer Institute (Yuspa *et al.*, 1980). Both transformed cell lines were grown in DME supplemented with 10% fetal calf serum, 100 units/ml penicillin and 100 mg/ml streptomycin.

Serum-free conditioned medium and cell lysates (serum-free) were prepared as follows. Confluent monolayers were washed two times with PBS and once with DME. Cells were then incubated with DME (8 ml per flask) for 24 h. This conditioned medium was removed, centrifuged and stored at −70 °C. The cells were then washed three times with PBS. Cell lysates were prepared using 0·01 M Tris, pH 7·5 with 0·5% Triton X-100 and 0·02 M $MgCl_2$ (2 ml/flask) for 5 min on ice. The cells were scraped from the flask with a rubber 'policeman' and centrifuged. Supernatants were stored at −70°C.

Electrophoretic analysis of plasminogen activators in SDS-polyacrylamide gels

This technique is based on a modification of the method of Heussen & Dowdle (1980). SDS-gel electrophoresis was carried out in slab gels using a 12·5% polyacrylamide running gel and 5% polyacrylamide stacking gel prepared as described by Anderson, Baum & Gesteland (1973). Human plasminogen and α-casein (Sigma, St. Louis, MO) were added to the gel preparation solutions before polymerization at a final concentration of 20 g/ml and 0·1%, respectively. The buffer system used was that of Laemmli (1970). Sample buffer was added to conditioned medium and cell lysates to give a final concentration of 0·06 M Tris, pH 6·8; 2·3% SDS (w/v, Bio-Rad Laboratories, Richmond, CA); 10% glycerol and 0·005% bromphenyl blue (Bio-Rad). PA activity in each sample was determined using a ^{125}I–fibrin plate assay as described by Strickland & Beers (1976). Following electrophoresis the gel was shaken gently at room temperature for 1 h with 2·5% Triton X-100 in water to remove SDS, transferred to a bath containing 0·1 M glycine–NaOH, pH 8·3, and incubated at 37°C for 3–5 h.

Human plasminogen was prepared from human plasma by lysine–sepharose affinity chromatography (Deutsch & Mertz, 1970). Highly purified human urokinase was obtained from Leo Pharmaceuticals and used as a standard.

Extraction of plasminogen activator from epidermis

Normal adult human skin was obtained immediately after surgery. After removal of fatty tissue, the skin was cut into strips approximately 5 mm wide and incubated in 2 M KBr at 37°C for 1–2 h. The epidermis was then peeled off with forceps, washed with PBS three times, blotted on filter paper and weighed. PBS (0·1 M pH 7·0) was added to give a final w/v of 150 mg/ml. These preparations were frozen and thawed five times, agitated at 4°C for 2 h and centrifuged at 27,000 × ***g*** for 30 min. The supernatant was removed and the same volume of PBS containing 2 M KCl was added to the pellet. This mixture was agitated at 4°C overnight and centrifuged at 27,000 × ***g*** for 30 min. The supernatant was collected and analysed following dialysis against 0·1 M Tris HCl pH 8·1 at 4°C.

RESULTS AND DISCUSSION

Plasminogen activators are detected as transparent bands using this electrophoretic technique (Fig. 1). The use of α-casein instead of gelatin enhanced the sensitivity of the method two-fold. Furthermore, casein did not migrate appreciably in the gel matrix which gave more reproducible results. The major bands of plasminogen activators detected in this gel system in culture medium from human neonatal epidermal cells, human adult epidermal cells and ME 180

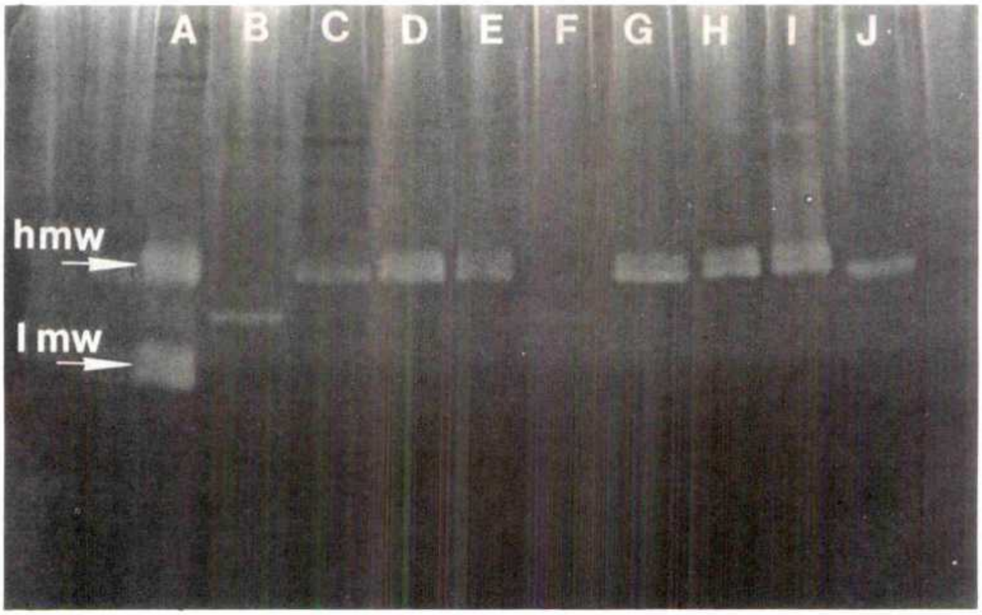

FIGURE 1. Electrophoresis of secreted and cell-associated plasminogen activators. Samples of conditioned medium or cell lysates were run in SDS-PAGE gels into which casein and plasminogen were co-polymerized. (A) urokinase standard, (B) PAM cell lysate, (C) ME 180 cell lysate, (D) cultured human adult epidermal cell lysate, (E) cultured human neonatal epidermal cell lysate, (F) PAM conditioned medium, (G) ME 180 conditioned medium, (H) cultured human adult epidermal cell conditioned medium, (I) cultured human neonatal epidermal cells conditioned medium, (J) extract of adult human epidermis. Total amounts applied ranged from 100 to 500 Ploug milliunits of urokinase equivalent activity. hmw = high molecular weight; lmw = low molecular weight.

cells migrated as a doublet the same distance as the high molecular weight band of human urokinase which was reported by Lesuk *et al.* (1965) and by White, Barlow & Mozen (1966) to have a molecular weight of 55,000 daltons. In medium and lysates from neonatal and adult human epidermal cells there is some trailing of enzymatic activity with an occasional minor higher molecular weight band. In contrast, the major band of plasminogen activator detected in medium from PAM cells migrated between the high molecular weight band of urokinase and low molecular weight urokinase, indicating that plasminogen activators of mouse epidermal cells are different from those of human epidermal cells. Plasminogen activators detected in the cell lysates of all cell cultures co-migrated with the major band found in the culture medium. Plasminogen activator extracted from normal adult human epidermis migrated with the major band of plasminogen activator detected in cultured neonatal and adult epidermal cells and ME180.

Wilson *et al.* (1980) classified plasminogen activators released by human cultured cells into two groups, of mol. wt around 55,000 (urokinase type) and 70,000 (melanoma type) daltons. These investigators defined plasminogen activators released by most cultured human epithelial cells (neonatal and adult) as predominantly of a urokinase type. Our observation is consistent with their result. We observed that the migration of plasminogen activator extracted from epidermis was similar to that of plasminogen activators of cultured epidermal cells, confirming the usefulness of cultured epidermal cells for the study of plasminogen activator in epidermal physiology. Wilson *et al.* (1980) and Heussen & Dowdle (1980) reported slightly higher molecular weights for the major plasminogen activators released in culture medium by cultured foreskin epithelial cells, but exact molecular weights cannot be determined using protein molecular weight standards because of anomalous electrophoretic behaviour of unheated, unreduced proteins in SDS-PAGE (Evers *et al.*, 1982). Our observations suggest that the molecular weight of the major plasminogen activator released by cultured epidermal cells is approximately 55,000 since its electrophoretic mobility is identical to the high molecular weight band of authentic urokinase which has a molecular weight of 55,000 daltons (Lesuk *et al.*, 1965). It is noteworthy that Wilson *et al.* (1980) and Heussen & Dowdle (1980) reported that the major bands of plasminogen activators from cultured epidermal cells migrate as a doublet. Fig. 1 confirms these observations. The cause for the doublet has not been defined but minor alterations in glycosylation or amino acid composition are likely explanations (Vetterlein *et al.*, 1980). The minor higher molecular weight bands may represent 'melanoma' or 'tissue factor' type P.A or they might reflect aggregates of plasminogen activators.

The molecular weights of plasminogen activators released into culture medium by normal mouse epidermal cells have been reported as 90,000, 88,000, 39,000 and 36,000 (Brown, 1982). These data as well as our own observations with PAM cells indicate that plasminogen activators in mouse cells are different from those in human epidermal cells. Species variations in plasminogen activators have been reported (Granelli-Piperno & Reich, 1978).

Using the caseinolysis gel the major plasminogen activators produced by human epidermal cells in culture are of the urokinase type and these enzymes are similar to those detected in human epidermal extracts. Human epidermal cell cultures and the human epithelial cell line ME 180 are suitable models to study modulation of plasminogen activator activity in experimental situations.

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Periodate-labile structures at the normal human cutaneous basement membrane zone*

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SUMMARY

The distribution of carbohydrates containing 1,2-glycol groups at the basement membrane zones of the dermo-epidermal junction and dermal blood vessels was investigated using the periodic acid-thiosemicarbazide-silver proteinate technique. Controls included pre-incubation with dimedone, dimedone treatment both before and after periodate oxidation, and dimedone treatment followed by thiosemicarbazide and silver proteinate.

At the dermo-epidermal junction the anchoring fibrils were stained selectively after both short (45 min) and long (71 h) thiosemicarbazide incubation periods, there being no staining of the lamina densa; however, the lamina densa surrounding blood vessels was labelled. After prolonged treatment with thiosemicarbazide, fibrils were observed traversing the lamina lucida at the dermo-epidermal junction, and collagen fibres were stained faintly. Pre-incubation with dimedone to block aldehyde groups did not affect the staining. However, when periodic acid treatment was omitted, or when sections were incubated with dimedone both before and after periodate oxidation, only melanin granules and the cytoplasm of fibroblasts were stained.

Thus, anchoring fibrils are rich in carbohydrates containing 1,2-glycol groups susceptible to periodate oxidation, and correspond to the periodic acid Schiff-positive layer seen at the dermo-epidermal junction. Furthermore, the basement membrane zones of dermal blood vessels and the dermo-epidermal junction differ in their carbohydrate distribution.

It has long been known that at the dermo-epidermal junction there is a thin layer which is stained by the periodic acid-Schiff and related techniques. This layer has been called the 'basement membrane' but it is not clear to which ultrastructural components this periodate-labile zone corresponds.

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Swift & Saxton (1967) used a periodic acid-silver methenamine technique at the electron microscopic level to show that, in human scalp and monkey gingiva, the periodate-labile zone is the superficial layer of the dermis and does not involve the lamina lucida or the lamina densa. A similar technique applied to the human ectocervix (Younes *et al.*, 1965) stained the anchoring fibrils and collagen fibres in the 0·5 μm area of stroma immediately subjacent to the lamina densa. However, in Rhesus monkeys, the lamina densa at the dento-epithelial junction and at the adjacent epithelio-connective tissue interface was stained by the periodic acid-silver methenamine and by the periodic acid-thiosemicarbazide (TSC)-silver proteinate reactions (Kobayashi, Rose & Mahan, 1977). Furthermore, the periodic acid-silver methenamine technique, when applied to a variety of other tissues, stained the lamina lucida and the lamina densa (Rambourg & Leblond, 1967).

The periodic acid-TSC-silver proteinate method (Thiéry, 1967) is a modification of the procedure introduced by Hanker *et al.* (1964), where osmium tetroxide was used to demonstrate bound TSC. An important factor in the specificity of the procedure is the incubation period with the TSC. Thus, for Araldite-embedded material glycogen could be demonstrated after 30–40 min incubation with TSC, mucopolysaccharides required 24–48 h, whilst glycoproteins needed 48–72 h (Thiéry, 1967). As this method gives excellent ultrastructural resolution, it has been used in the present study to determine the periodate-labile structures in normal human skin.

METHODS

Normal skin was obtained from the abdomen of a 29-year-old Caucasian woman and the breast of a 19-year-old female Negro patient whilst undergoing plastic surgery, and was fixed in 4% paraformaldehyde in 0·1 mol/l cacodylate buffer (pH 7·2). It was then dehydrated through a series of ethanols and embedded in Araldite. Free-floating ultrathin sections were stained by the technique described by Thiéry (1967). The sections were oxidized for 25 min on 1% aqueous periodic acid, washed with distilled water and incubated at 20°C with 1% thiosemicarbazide (TSC) in 10% acetic acid for 45 min or 71 h. They were then washed with successively decreasing concentrations of acetic acid followed by distilled water, floated on 1% aqueous silver proteinate for 30 min at 20°C in the dark, rinsed thoroughly with distilled water, picked up on grids and viewed without further counterstaining.

In addition, three control procedures were performed.

Control 1. Pre-existing aldehyde groups were blocked by treating the sections with a saturated solution of dimedone (5,5-dimethyl-1,3-cyclohexanedione) in 1% acetic acid for 2 h at 60°C. The staining sequence was dimedone–periodic acid–TSC–silver proteinate.

Control 2. The sections were treated with dimedone both before and after periodate oxidation, followed by TSC and silver proteinate.

Control 3. The staining sequence was dimedone–TSC–silver proteinate.

RESULTS

The ultrastructure of the tissue was well preserved (Fig. 1) by the fixation and embedding procedures employed. Glutaraldehyde was not used because of the theoretical risk of

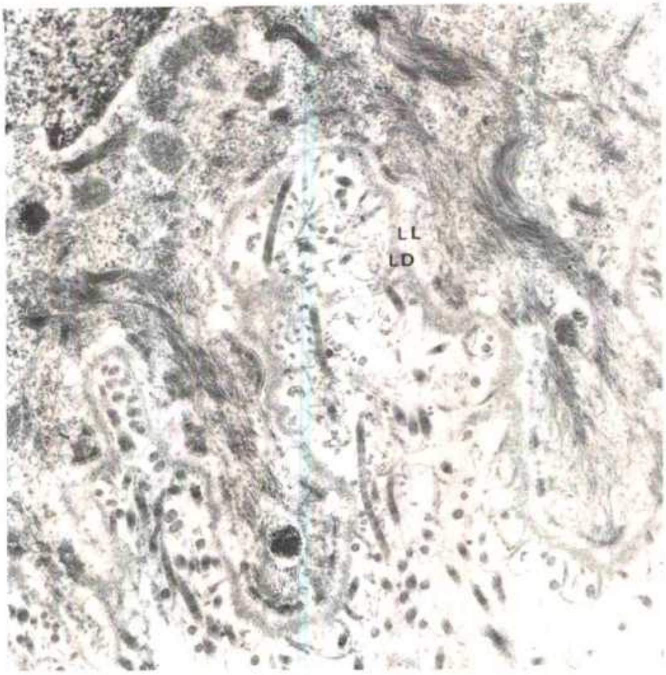

FIGURE 1. Normal dermo-epidermal junction. The tissue ultrastructure is well preserved. LL = lamina lucida; LD = lamina densa. All the micrographs demonstrate staining of tissue obtained from the Caucasian patient. (Uranyl acetate and lead citrate, × 22,820).

introducing free aldehyde groups, a problem not encountered with paraformaldehyde if the tissue is washed thoroughly after fixation. The omission of post-fixation with osmium tetroxide resulted in the extraction of lipids, but osmium tetroxide reacts with 1,2-glycol groups (Wolman, 1955) and has been shown to interfere with the staining procedure in certain instances (Thiéry, 1967). Identical results were obtained with tissue from both patients on staining with periodic acid-TSC-silver proteinate.

TSC treatment for 45 min

At the dermo-epidermal junction (Fig. 2) the anchoring fibrils were stained, there being no silver deposited at the lamina densa. Granular deposits of silver were present in the cytoplasm of both epidermal cells and fibroblasts, but they were much larger in the latter and existed in all fibroblastic processes. In addition, melanin granules were labelled.

The lamina densa surrounding the dermal blood vessels took up the silver (Fig. 3) and there was some cytoplasmic staining of inflammatory and endothelial cells; the surfaces of the latter were also labelled.

TSC treatment for 71 h

With prolonged TSC incubations (Fig. 4) the anchoring fibrils were again stained, but tenuous fibrils were observed traversing the lamina lucida, and at hemi-desmosomes the intermediate dense lines were quite prominent. Collagen fibres were labelled faintly, showing their characteristic striations. Occasionally, the plasma membranes of epidermal cells took up the

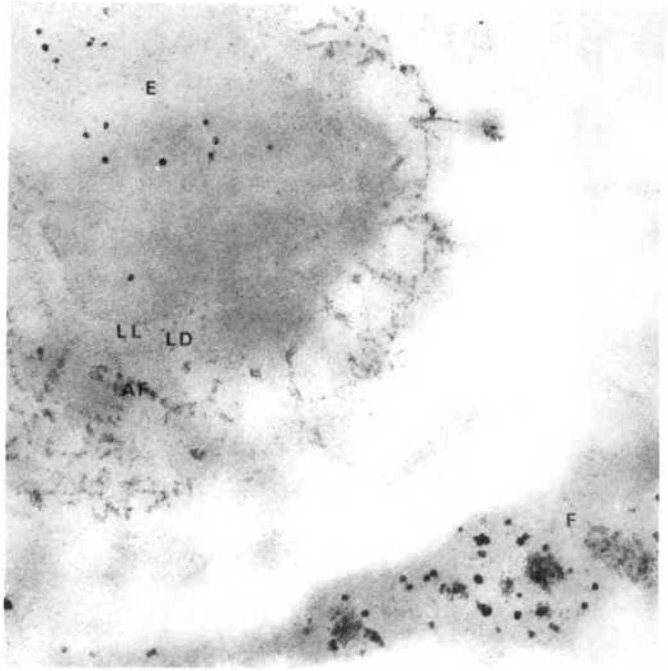

FIGURE 2. The anchoring fibrils (AF) are stained, but there is no staining of the lamina lucida (LL) or the lamina densa (LD). Granular silver deposits are present in fibroblast (F) and epidermal (E) cytoplasm. (Periodic acid-thiosemicarbazide (45 min)-silver proteinate, × 47,600).

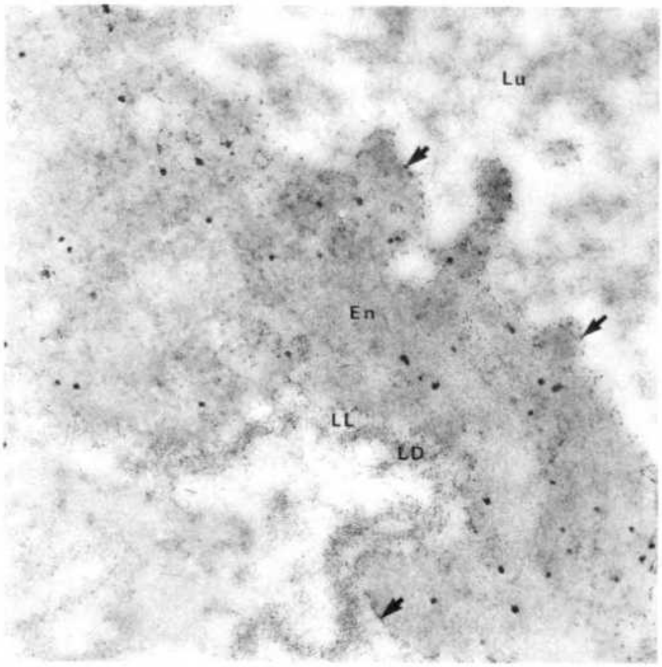

FIGURE 3. Wall of dermal blood vessel. The lamina densa (LD) is stained, but the lamina lucida (LL) remains unstained. The surfaces of the endothelial cell (En) are labelled (arrows). Lu = vessel lumen. (Periodic acid-thiosemicarbazide (45 min)-silver proteinate, × 47,600).

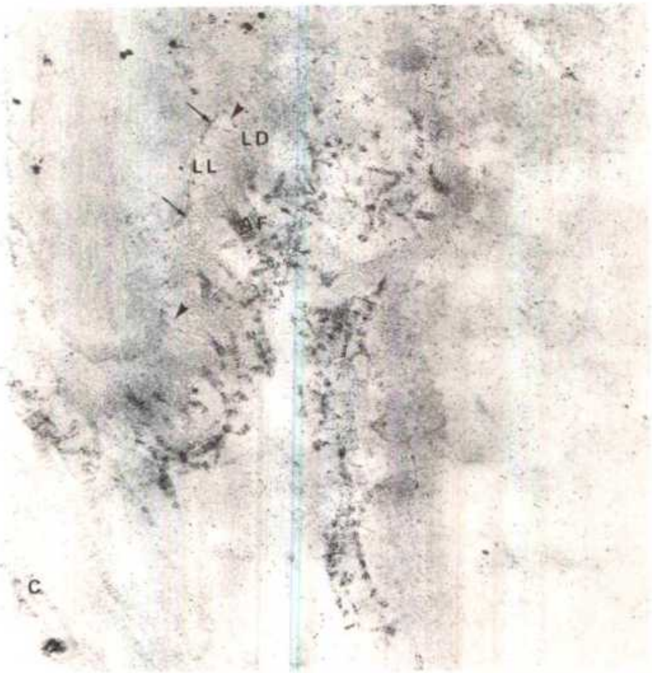

FIGURE 4. The anchoring fibrils (AF) are stained, but there is no staining of the lamina densa (LD). Tenuous fibrils (arrowheads) are traversing the lamina lucida (LL) and the intermediate dense lines (arrows) of hemi-desmosomes are apparent. Collagen fibres (C) are faintly stained. (Periodic acid-thio-semi-carbazide (71 h)-silver proteinate, × 47,600).

stain and sometimes delicate reticular networks of silver particles were deposited over the nuclei. Dense silver deposits were present over melanin granules and the cytoplasm of fibroblasts.

The lamina densa of dermal blood vessels was again stained, but the labelling of endothelial cell surfaces and cytoplasm was more prominent than after 45 min incubation with TSC.

Control 1

The staining pattern after this procedure was identical to that obtained when dimedone treatment was omitted.

Control 2

In this case there was no staining of the basement membrane zone but the staining of melanin granules and the cytoplasm of fibroblasts persisted.

Control 3

Again, melanin granules and the cytoplasm of fibroblasts were the only structures stained.

DISCUSSION

The periodic acid-TSC-silver proteinate technique provided excellent ultrastructural definition and it has been suggested that it is more specific for 1,2-glycol groups than the periodic acid-silver methenamine reaction (Courtoy & Simar, 1974).

The lack of staining at the basement membrane zone when sections were treated with dimedone, both before and after periodate oxidation (Control 2), showed that the staining of all structures, except for melanin granules and the cytoplasm of fibroblasts, was indeed due to 1,2-glycol groups oxidized by periodic acid. Pre-treatment of the sections with dimedone (Control 1) did not affect the staining, indicating that no free aldehyde groups pre-existed in the material. Thus, the paraformaldehyde fixative employed not only provided good ultrastructural preservation, but did not introduce any artefactual staining and permitted excellent penetration of the reagents. The results of the third control procedure (dimedone-TSC-silver proteinate) showed that the dimedone treatment effectively blocked all free aldehyde groups in the material. The staining of melanin granules and of the cytoplasm of fibroblasts was non-specific in that it was not due to the presence of 1,2-glycol groups.

With both short (45 min) and long (71 h) TSC incubation periods anchoring fibrils were stained, suggesting that they are rich in carbohydrates containing 1,2-glycol groups susceptible to periodate oxidation and are probably responsible for the distinctive staining pattern observed after the periodic acid-Schiff and related techniques. Although the lamina densa at the dermo-epidermal junction was always unlabelled, it stained around dermal blood vessels. This indicates that there is a fundamental chemical difference between the structures at the basement membrane zones of the two sites, and explains the apparently contradictory data of previous workers (Swift & Saxton, 1967; Rambourg & Leblond, 1967). The tenuous fibrils observed at the dermo-epidermal lamina lucida were especially numerous at hemidesmosomes and probably corresponded to anchoring filaments.

The glycoprotein nature of collagen fibres was revealed by their faint, but distinctive, staining after 71-h treatment with TSC, and the labelling of some cellular membranes was probably due to membrane-associated glycoproteins.

It is concluded that anchoring fibrils are rich in carbohydrates containing 1,2-glycol groups susceptible to periodate oxidation, and that the components of the basement membrane zone at the dermo-epidermal junction are biochemically distinct from those surrounding dermal blood vessels. It would be of interest to see whether the staining characteristics of these structures are altered in pathological conditions such as epidermolysis bullosa dystrophica.

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Immunofluorescence studies in progressive systemic sclerosis (scleroderma) and mixed connective tissue disease

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SUMMARY

Immunofluorescence (IF) investigations of the skin were performed in thirty patients with progressive systemic sclerosis (scleroderma) and eight patients with mixed connective tissue disease (MCTD). The results show that speckled epidermal nuclear immunoglobulin deposition occurs not only in MCTD but also in true scleroderma. Granular IgM deposition at the dermo-epidermal junction of light-exposed skin was detected in both groups of patients, but six of eight MCTD patients also showed a granular IgM band in non-exposed skin.

Antinuclear antibodies (ANA) were demonstrated in the sera of 96% and 100% of patients with scleroderma and MCTD respectively. The pattern of nuclear IF staining in scleroderma included dense fine speckles, large coarse speckles, threads, nucleolar and centromere staining. In MCTD, by contrast, the ANA staining pattern consisted of threads. The significance of ANA titres and immunological specificities for the *in vivo* reaction of serum ANA with epidermal nuclear antigens is discussed.

Cutaneous immunofluorescence in patients with connective tissue diseases is of diagnostic value (Beutner, Chorzelski & Bean, 1979). Epidermal nuclear immunoglobulin deposition most frequently occurs in systemic lupus erythematosus (Tan & Kunkel, 1966; Shu *et al.*, 1977; Wells *et al.*, 1979) and mixed connective tissue disease (Gilliam, Smiley & Ziff, 1975; Gilliam & Prystowsky, 1977; Prystowsky & Tuffanelli, 1978; Bentley-Phillips & Geake, 1980). Patients with speckled epidermal nuclear immunoglobulin deposits usually reveal high titres of serum ANA against n-RNP, a fraction of saline-extractable nuclear antigens (ENA). From these findings it was concluded that speckled nuclear immunoglobulins in the epidermis can be

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TABLE 1. Clinical data and immunofluorescence findings in patients with progressive systemic sclerosis

					Epidermal nuclear Ig deposition			Granular Ig at DEJ/ vessels (V)/cytoid bodies (CB)			ANA titres and staining patterns		
Case	Sex	Age (yrs)	Duration of symptoms (yrs)	Scleroderma variant	IgG	IgA	IgM	IgA	IgM	C3	IgG	IgA	IgM
1	F	54	2	CREST	+	–	– }	*	DEJ/CB		1:8192	—	1:128
					+	–	– }		CB		(ls)		(sp)
2	F	55	15	Diffuse scleroderma	–	–	–		DEJ/CB		1:4096	—	1:256
					–	–	–		CB		(sp)		(sp)
3	F	53	13	CREST	–	–	–				1:4096	—	1:64
					–	–	–				(sp)		(sp)
4	F	61	17	CREST	+	–	–		DEJ		1:4096	1:128	1:512
					+	–	–		CB		(th)	(sp)	(sp)
5	M	39	5	CREST	–	–	–				1:32	—	1:64
					–	–	–				(ds)		(sp)
6	F	78	5	CREST	–	–	–		DEJ		1:512	—	—
					–	–	–				(ls)		
7	M	56	2	Diffuse scleroderma	+	–	–		DEJ/CB/V		1:1024	—	1:128
					+	–	–				(sp)		(sp)
8	F	34	1	CREST	–	–	–		DEJ V		1:32	—	—
					–	–	–		CB		(ds)		
9†	M	58	3	Diffuse scleroderma	+	+	–	CB	DEJ/CB		1:512	1:1024	1:64
					+	+	–				(sp)	(sp)	(sp)
10	F	42	20	CREST	–	–	–		DEJ		1:64	1:128	1:64
					–	–	–				(sp)	(sp)	(sp)
11	M	37	1	Diffuse scleroderma	+	–	–		DEJ		1:4096	—	—
					+	–	–				(nu)		
12	M	35	2	CREST	–	–	–		DEJ V		—	—	1:32
					–	–	–						(sp)
13	F	54	14	Diffuse scleroderma	–	–	–		DEJ		1:256	—	1:64
					–	–	–				(sp)		(sp)
14	F	55	8	CREST	+	–	–				1:4096	—	1:256
					+	–	–				(nu)		(sp)

(cont'd. on p. 29)

15	F	29	10	Diffuse scleroderma	– –	– –	– –		CB	1:4096 (sp)	1:128 (sp)	1:128 (sp)
16	F	59	27	C R E S T	– –	– –	– –		CB	1:128 (ds)	—	1:512 (sp)
17	F	51	16	C R E S T	– –	– –	– –			1:128 (sp)	—	—
18	F	36	2	C R E S T	– –	– –	– –			1:32 (ds)	—	1:256 (sp)
19	F	57	10	Diffuse scleroderma	+ +	– –	– –		DEJ/CB/V	1:2048 (sp)	—	1:64 (sp)
20	M	56	11	C R E S T	– –	– –	– –		DEJ/CB/V	—	—	1:512 (sp)
21	M	36	4	C R E S T	– –	– –	– –			—	—	1:128 (sp)
22	M	47	5	C R E S T	– –	– –	– –		DEJ	1:64 (sp)	—	1:64 (sp)
23	F	44	5	Diffuse scleroderma	– –	– –	+ –		DEJ	1:1024 (sp)	— —	1:1024 (sp)
24	F	55	7	C R E S T	– –	– –	– –		DEJ	1:256 (th)	—	—
25	F	69	5	C R E S T	–	–	–		CB	—	—	—
26	F	45	10	Diffuse scleroderma	– –	– –	– –		DEJ	1:256 (sp)	—	—
27	F	47	10	C R E S T	– –	– –	– –			1:128 (sp)	—	—
28†	F	65	25	C R E S T	– –	– –	– –	CB	DEJ/CB	1:512 (sp)	—	1:256 (sp)
29	F	46	9	C R E S T	– –	– –	– –			1:32 (nu)	—	—
30	M	59	1	Diffuse scleroderma	+ +	– –	– –			1:2048 (sp)	—	—

* First line = sun-exposed; second line = sun-protected skin (each case).

† Patients receiving immunosuppressive treatment.

C = calcinosis; R = Raynaud's phenomenon; E = esophageal dysmotility; S = sclerodactyly; T = telangiectasia.

sp = dense fine speckles; ls = large coarse speckles; ds = discrete speckles (centromere staining); th = threads; nu = nucleolar staining.

TABLE 2. Clinical data and immunofluorescence findings in patients with mixed connective tissue disease

			Duration of		Epidermal nuclear Ig deposition			Granular Ig at DEJ/ vessels (V)/cytoid bodies (CB)			ANA titres and staining patterns		
Case	Sex	Age (yrs)	disease (yrs)	Clinical symptoms ANA specificity	IgG	IgA	IgM	IgA	IgM	C3	IgG	IgA	IgM
1	F	32	8	Scleroderma/rheumatoid arthritis/n-RNP	+	–	–		DEJ/CB/V		1:4096 (th)	—	1:64 (sp)
					+	–	–		DEJ				
2	F	60	4	Scleroderma/rheumatoid arthritis/n-RNP	+	+	–		DEJ V		1:8192 (th)	1:1024 (th)	1:512 (th)
					+	+	–		DEJ V				
3	F	43	9	Scleroderma/SLE/myositis/ rheumatoid arthritis/n-RNP	–	–	–		DEJ		1:512 (th)	—	1:128 (sp)
					–	–	–		DEJ				
4	F	39	6	Scleroderma/SLE/rheumatoid arthritis/n-RNP	+	+	–	CB	DEJ		1:8192 (th)	1:2048 (th)	1:4096 (th)
					+	+	–		DEJ				
5	F	60	18	Scleroderma/rheumatoid arthritis/n-RNP	+	+	+		DEJ	DEJ	1:8192 (th)	1:2048 (th)	1:4096 (th)
					+	+	+						
6	F	43	6	Scleroderma/myositis/rheumatoid arthritis/n-RNP	+	–	–		DEJ/CB		1:2048 (th)	—	1:128 (sp)
					+	–	–		DEJ/CB				
7	M	36	5	Scleroderma/myositis/rheumatoid arthritis/n-RNP	+	–	–		DEJ/CB	V	1:4096	—	1:512
					+	–	–						
8†	F	42	8	Scleroderma/SLE/rheumatoid arthritis/n-RNP/Sm	–	–	–		DEJ		1:512 (th)	—	1:64 (sp)
					–	–	–		DEJ				

The symbols are as in Table 1 (p. 29).

regarded as a hallmark of connective tissue diseases characterized by antibody to n-RNP. Most of these patients showed symptoms of mixed connective tissue disease as first described by Sharp and co-workers (1972).

Previous reports have documented the occurrence of granular immunoglobulin and/or complement deposition at the DEJ of uninvolved skin in MCTD (Winkelmann, Carapeto & Jordon, 1977; Gilliam & Prystowsky, 1977; Izuno, 1978; Wells *et al.*, 1979). In scleroderma, direct IF investigations both on indurated areas and apparently normal skin have been regarded as uniformly negative (Burnham, Fine & Neblett, 1970; Tuffanelli, 1975; Winkelmann *et al.*, 1977; Connolly & Winkelmann, 1981).

In the present study, direct IF investigations of skin biopsies from thirty scleroderma and eight MCTD patients were performed. The findings were correlated with corresponding serum ANA titres, n-RNP specificity and subtle immunofluorescent ANA staining patterns.

METHODS

The patients in this study were divided into two groups. The first consisted of thirty patients (twenty-one female and nine male) with clinical features characteristic of the diagnosis of progressive systemic sclerosis. Their mean age was 52 years (range 29–78). The mean duration of disease was 9 years (range 1–27). The second group consisted of eight patients with clinical symptoms and laboratory findings suggestive of the diagnosis of mixed connective tissue disease. There were seven females and one male with a mean age of 44 years (range 32–60). The mean duration of disease was 8 years (range 4–18). The investigations were performed with the informed consent of the patients.

Punch biopsies (4 mm) from sun-exposed (dorsum of wrist) and protected (inner aspect of upper arm) skin were immediately frozen in liquid nitrogen, stored at −70°C and processed within 1 week. Specimens were sectioned (4–6 μ) in a cryostat at −20°C, placed on a microscope slide, air dried for 15 min and used both unfixed and acetone-fixed for 10 min in the established IF staining procedure (Beutner *et al.*, 1979). A Leitz Orthoplan microscope with a Hg-50 W lamp and a Leitz Pleompak 2·1 vertical illuminator were used. FITC fluorescence was detected with the filter-block I 2 and NPL Fluortar oil immersion objectives. FITC-labelled monospecific goat antisera to human IgA, IgM, IgD, IgE and fibrinogen were obtained from Tago, Inc., Burlingame, U.S.A.; rabbit raised antisera to IgG, C1q, C3 and C4 from Behringwerke AG, Germany. The antisera were tested for specificity by single radial immunodiffusion or immunoelectrophoresis and used in optimal working dilutions.

ANA titres were assessed by serial dilutions of the patients' sera using acetone-fixed rat liver cryostat sections as substrate and FITC-labelled antiglobulins to IgG, IgA and IgM in optimal dilutions as determined by chessboard titration. Immunofluorescent ANA staining patterns for IgG, IgA and IgM were separately assessed using KB human epithelial tissue culture cells as substrate (provided by Electro-Nucleonics Lab., Inc., U.S.A.). Specificity of serum ANA for n-DNA was assessed by using the *Crithidia luciliae* substrate. ANA specificity for n-RNP and Sm antigen was determined by counter-immunoelectrophoresis (Institute of Immunology, University of Heidelberg, Germany).

RESULTS

Direct IF findings in the patients with scleroderma ($n = 30$) showed speckled epidermal nuclear IgG deposition in six cases. Additional IgA was observed in one patient. Speckled epidermal

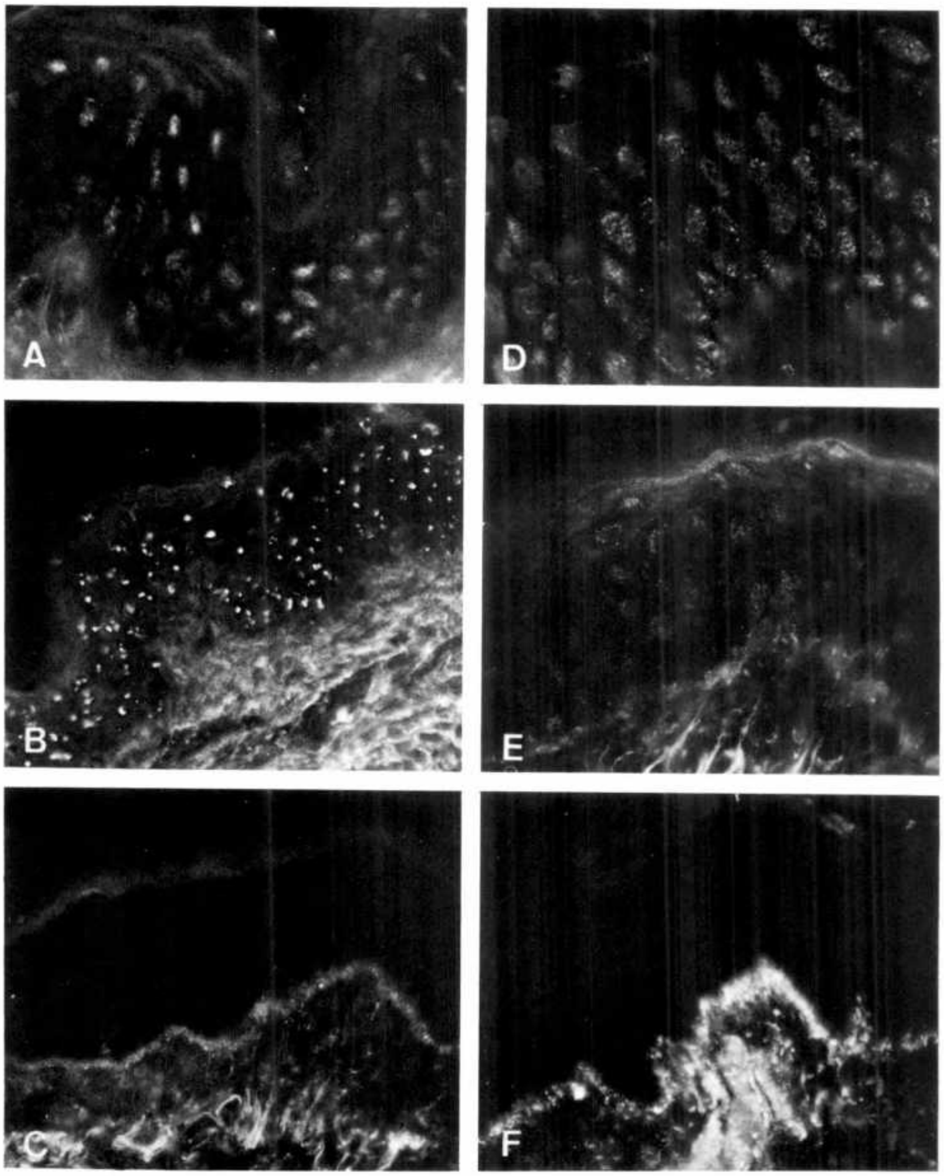

FIGURE 1. Direct immunofluorescence findings of the skin from scleroderma (A, B, C) and MCTD patients (D, E, F). Note speckled epidermal nuclear IgG deposition in both scleroderma (A) and MCTD (D). Nucleolar IgG was observed in scleroderma (B). Granular IgM deposits at the DEJ of sun-exposed skin was detected in scleroderma (C) and MCTD (F). Additional IgM at the DEJ of sun-protected skin was found in MCTD (E). Granular IgM in papillary vessel walls in MCTD is shown in (F). Note speckled IgM deposition in epidermal nuclei in MCTD (E). A, B and E represent biopsies from sun-protected skin, C, D and F from areas of sun-exposed skin. Magnification: A and E, × 265; B, C and F, × 210; D, × 530.

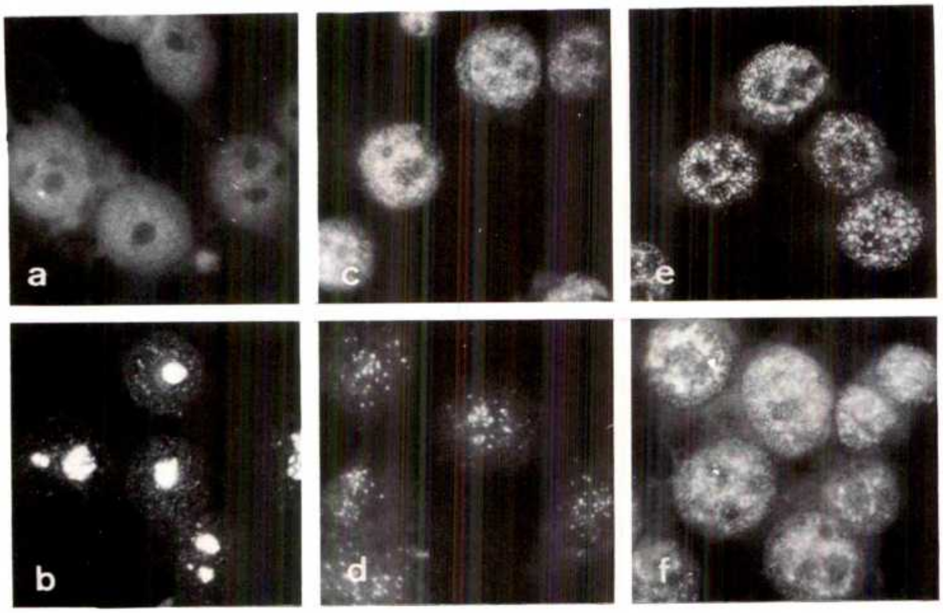

FIGURE 2. Immunofluorescent ANA staining patterns for IgG produced by scleroderma (a, b, c, d, e) and MCTD (f) sera. The fine speckles (a) are so dense that this pattern could be mistaken for homogenous staining. Nucleolar staining is represented in (b), threads of scleroderma sera in (c), large coarse speckles in (e). Discrete speckles of (d) are believed to be due to anti-centromere staining. Threads as observed in MCTD are demonstrated in (f). For evaluation of ANA staining patterms, the indirect immunofluorescence technique was used and KB oral epidermoid cell tissue cultures were the substrate. Magnification × 530.

nuclear IgM alone was detected in the sun-exposed skin from another patient. Nucleolar epidermal IgG was found in two patients. Granular IgM at the DEJ was present in the light-exposed skin from eighteen cases, granular IgM deposits in papillary vessel walls from five patients. Subepidermal cytoid bodies for IgA and/or IgM were observed in thirteen patients.

IgG-class serum ANA were detected in twenty-seven patients with a mean titre of 1:1024 (range 1:32 to 1:8192), IgA-class ANA in four patients with a mean titre of 1:256 (range 1:32 to 1:1024) and IgM-class ANA in twenty patients with a mean titre of 1:256 (range from 1:16 to 1:1024). Serum ANA of scleroderma patients were not reactive with n-RNP, Sm or N-DNA antigen.

Predominantly, immunofluorescent ANA staining patterns for IgG consisted of dense fine speckles (n = 15), large coarse speckles (n = 2), threads (n = 2), nucleolar staining (n = 3) and discrete speckles (n = 4) which is believed to be due to anti-centromere staining (Tan *et al.*, 1980).

Speckled epidermal nuclear IgG was detected in six MCTD patients. Additional IgA was observed in three cases, and IgM in one patient. Granular IgM at the DEJ of sun-exposed skin was demonstrated in all MCTD patients at an intensity usually higher than observed in the scleroderma group. In addition, six out of eight cases showed a granular IgM band at the DEJ of their sun-protected skin. Granular IgM in papillary vessel walls was observed in three patients, granular C3 in one case. Deposition of granular C3 at the DEJ was present in another patient.

Subepidermal cytoid bodies for IgA and/or IgG were seen in three patients. IgG- and IgM-class ANA were detected in the sera of all MCTD patients.

Mean ANA titre for IgG was 1:4096 with a range from 1:512 to 1:8192, for IgM 1:1024 with a range from 1:64 to 1:4096. IgA-class ANA were detected in three patients with a mean titre of 1:1024 and a range from 1:1024 to 1:2048. All MCTD patients showed serum ANA with specificity for n-RNP. One patient in addition revealed ANA specificity for Sm antigen. ANA with specificity for n-DNA were not observed. ANA staining patterns of IgG-class ANA were fine to large threads in all eight cases.

In scleroderma patients with epidermal nuclear IgG staining, mean serum ANA titre of IgG-class was 1:2048 with a range from 1:512 to 1:8192. Mean IgG-class ANA titre in scleroderma patients without epidermal nuclear staining was 1:256 with a range from 1:16 to 1:4096. Statistical comparison using the Wilcoxon–Mann–Whitney test revealed a significant difference ($P < 0{\cdot}05$).

The results of direct IF, serum ANA titres and immunofluorescent ANA staining patterns are displayed in Tables 1 and 2 as well as Figs 1 and 2.

DISCUSSION

Several findings of the present study are in good agreement with those of Gilliam *et al.* (1975), Prystowsky & Tuffanelli (1978), Wells *et al.* (1979), Bentley-Phillips & Geake (1980) and Nunzi, Parodi & Rebora (1982). In particular, we confirmed the occurrence of speckled epidermal nuclear IgG deposition in MCTD patients with high titres of serum ANA against n-RNP antigen. We also demonstrated a granular IgM band at the DEJ of indurated skin as described by Winkelmann *et al.* (1977). In addition, we found a granular IgM band at the DEJ in the sun-protected uninvolved skin from six of eight patients. We consider the latter finding to be of diagnostic significance since it was not observed in our patients with scleroderma. However, a large number of scleroderma patients showed weak granular IgM deposits at the DEJ of their sun-exposed skin. These findings can be regarded as non-specific (Jablonska, Chorzelski & Maciejowska, 1970).

The present study demonstrates the occurrence of speckled epidermal nuclear IgG, IgA or IgM deposition in seven of thirty scleroderma patients. These findings are in contrast to previous reports on direct immunofluorescence in scleroderma which were negative (Burnham *et al.*, 1970; Tuffanelli, 1975; Winkelmann *et al.*, 1977; Connolly & Winkelmann, 1981). On the other hand, we confirmed deposition of nucleolar IgG in the epidermis of two of our scleroderma patients as recently described by Prystowsky, Gilliam & Tuffanelli (1978).

There is still controversy as to whether epidermal nuclear immunoglobulin deposition in connective tissue disease is an *in vivo* reaction of serum ANA with epidermal nuclear antigens or simply a technical artifact. Gilliam (1975) felt it was artifact produced during biopsy-taking or processing for direct IF. Tuffanelli (1975), Izuno (1978) and Wells *et al.*, (1979) thought it was an *in vivo* reaction. This view is supported by findings of Alarcón-Segovia, Ruiz-Argüelles & Fishbein (1979) who demonstrated that n-RNP antibodies are capable of penetrating viable blood mononuclear cells in MCTD. To explain ANA penetration into living cells in MCTD, Tan (1976) and Izuno (1978) postulated permeability-enhancing factors in the patients' sera.

In the present study, we also looked for factors possibly accounting for the *in vivo* reaction of serum ANA to epidermal antigens in scleroderma. We found a highly significant correlation between levels of serum ANA and the occurrence of epidermal nuclear staining. High titres of serum ANA against n-RNP are also a constant finding in MCTD. However, in our study, individual patients with scleroderma who showed moderate ANA titres (1:512), were also positive whereas others presenting high ANA titres (1:4096) were negative.

We conclude that permeability-enhancing factors may play an important role for the *in vivo* reaction of serum ANA to epidermal nuclear antigens in scleroderma. Furthermore, these factors can explain discrepancies of direct IF findings in scleroderma patients with apparently identical ANA titres and staining patterns. The nature of these permeability factors is still unknown and will require further clarification.

As a rule, only antibodies to n-RNP have been regarded as capable of producing epidermal nuclear staining *in vivo*. However, there have been observations on individual patients with scleroderma presenting speckled epidermal nuclear IgG deposition without showing serum ANA of n-RNP specificity (Wells *et al.*, 1979). Furthermore, the occurrence of nucleolar epidermal IgG is well documented (Prystowsky *et al.*, 1978). These reports demonstrate that ANA with a specificity different from n-RNP are able to react with the corresponding epidermal antigens as detected by direct IF. Our present findings confirm epidermal nuclear deposition of ANA without n-RNP specificity in scleroderma.

Antinuclear antibodies have been detected in the sera of 96% of our scleroderma and 100% of MCTD patients. These findings are in keeping with previous results on the prevalence of circulating ANA in scleroderma (Rothfield & Rodnan, 1968; Tan *et al.*, 1980) and mixed connective tissue disease (Gilliam & Prystowsky, 1977; Prystowsky & Tuffanelli, 1978). It is noteworthy that all our MCTD patients showed ANA of n-RNP specificity in contrast to our scleroderma group. Similar findings were made by Grennan *et al.* (1977) though Kurata & Tan (1976) and Maddison, Mogovero & Reichlin (1978) had shown that n-RNP antibodies are not strictly confined to MCTD but occur in scleroderma in low frequencies.

Immunofluorescent ANA staining patterns for IgG in scleroderma patients of this study predominantly consisted of dense fine speckles. The nature of the reactive antigen may be the non-histone nuclear protein termed Scl-70 (Douvas, Achten & Tan, 1979). The other patterns observed in scleroderma were large coarse speckles and nucleolar staining, the reactive antigen of the latter partly being 4-6 S nucleolar RNA (Pinnas, Northway & Tan, 1973). A fourth staining pattern consisted of discrete speckles easily distinguished. This pattern is believed to be due to anti-centromere staining as described by Tan *et al.* (1980). By contrast, our MCTD sera produced a uniform staining pattern consisting of fine to large threads.

In conclusion, direct immunofluorescence in our scleroderma patients has shown that speckled epidermal nuclear immunoglobulin deposition is not confined to mixed connective tissue disease with n-RNP specificity of serum ANA but also occurs in true scleroderma.

ACKNOWLEDGMENT

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The value of bacteriology and serology in the diagnosis of necrotizing fasciitis

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SUMMARY

Fourteen patients with necrotizing fasciitis are described. In thirteen the cause was *Streptococcus pyogenes* [Group A beta haemolytic streptococcus (BHS)]; in the fourteenth, *Staphylococcus aureus* was responsible. In the acute fulminating form of the disease, BHS can be cultured from the affected tissues. In the less acute form, particularly when the patient has been previously treated with antibiotics, other bacteria colonize the tissues and the BHS cannot be isolated. Serological evidence of infection with *Streptococcus pyogenes* can be ascertained in all such patients by finding high levels of anti-desoxyribonuclease B and anti-hyaluronidase. Measurement of the anti-streptolysin O titre is not helpful. Once the diagnosis is made, surgical removal of all necrotic tissue is still the treatment of choice.

Necrotizing fasciitis is a distinct clinical entity usually due to *Streptococcus pyogenes*. It was accurately described nearly 60 years ago (Meleney, 1924) but still goes unrecognized. Over a 9-month period between October 1980 and June 1981, five patients with necrotizing fasciitis were described in the *British Medical Journal*, all apparently undiagnosed. Four of these patients died very quickly (Redding, 1980; Cruickshank *et al.*, 1981) and the fifth lost a finger (Hawkey, Pedler & Southall, 1980). It appears that this variety of streptococcal infection in the skin is more common than realized. In the last 2 years we have seen fourteen such patients. The first three were recognized late and treated only with large doses of penicillin (Seal *et al.*, 1980). All three survived, but each had a long and stormy hospital course and later needed reconstructive surgery. One patient was admitted to hospital, but immediately became septicaemic and died within 24 h (patient 6). The other ten were recognized early and treated by surgical debridement as soon as the diagnosis was made. This resulted in considerably less time in hospital and no long-term morbidity.

In order to facilitate early diagnosis we looked at the results of bacterial cultures and the serological responses to infection to investigate whether either or both were helpful.

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TABLE 1. Clinical, bacteriological and serological details of the patients

Patient	Age	Sex	Site	Bacteriology (blister fluid/ necrotic tissue)	ASO§	Anti-DNAase B‡	Anti-hyaluronidase‡	ESR (mm in 1st hr)	WBC ($\times 10^9$/l)
Group 1									
1	56	M	Arm	Group A BHS (M_1, T_1)	90	1200	128	48	10·1
2	73	M	Leg	Group A BHS (M_2, T_2)	560	>9600	1024	150	20·3
3	43	M	Arm	Group A BHS (M_3, $T_3/_{12}/B_{3264}$)	180	600	128	59	9·2
4	72	F	Arm	Group A BHS (M_{-}, T_3)	800	6400	1024		
5	30	M	Leg	Group A BHS (M_{-}, T_{14})	90	>4096	2400	125	13·1
6	72	M	Leg	Group A BHS (M_{22}, T_{12})	160†	<50†	64†		6·3
7*	45	M	Face	Group A BHS (M_{75})	80	1200	128		19·7
8*	71	F	Face	Group A BHS (M_{75})	360	>9600	512	122	10·7
Group 2									
9*	41	M	Arm	No growth	320	2400	2048	104	18·2
10	68	F	Foot	*Streptococcus faecalis* and *Proteus sp.*	240	1200	1024	98	8·0
11	72	F	Leg	*Staphylococcus aureus*	280	6400	512	32	6·1
12	49	F	Leg	Group G BHS	280	1200	128 (Gp G = 10)	63	9·9
13	77	F	Leg	No growth	320	4800	<32	127	22·2
Group 3									
14	79	F	Leg	*Staphylococcus aureus*	110	250	110	91	10·1
				Highest normal limit	160	250	128 (Gp G = 20)		

* Seal *et al.* (1980)

† Acute serum only available

‡ Group A specific

§ Common to all haemolytic streptococci

PATIENTS AND METHODS

After the first three patients had been seen (Seal *et al.*, 1980; patients 7, 8 & 9 in Table 1), all local general practitioners were asked to let one of us (B.J.L.) know if they had any patient under their care with erysipelas, cellulitis or anything that looked similar. We asked if such patients could be admitted to the dermatology ward where a tentative clinical diagnosis would be made. If the diagnosis of necrotizing fasciitis was suspected, then bacteriological cultures were made from blister fluid or necrotic tissue, and from the patient's blood. *Streptococcus pyogenes* was identified by beta haemolysis on blood agar, and was typed as Lancefield Group A by a co-agglutination method. The serological response to streptococcal infection in the form of anti-streptolysin O (ASO) anti-desoxyribonuclease B (ADB) (Nelson, Ayoub & Wannamaker, 1968) and anti-hyaluronidase (AHT) (Murphy, 1972) was measured on admission, and again at 2 weeks and 3 months. As soon as the diagnosis of necrotizing fasciitis was made, surgical debridement was carried out and the necrotic tissue sent for bacteriological culture and histological examination. Routine paraffin-embedded sections were stained with haematoxylin and eosin, Gram stain and a concentrated ammonium oxalate Gram stain.

RESULTS

Clinical features

It soon became apparent that there was a spectrum of clinical disease which we have divided into three groups for clarity.

In the first group (patients 1–8), the patients were acutely and severely ill and without surgical intervention they might well have died (as did patient 6). All were admitted urgently to hospital; none had received previous antibiotic therapy at home.Two patients (5 and 7) were diabetic. None was receiving systemic steroids or immunosuppressive drugs.

Case history—patient 1. A previously fit 56-year-old male cut his right thumb whilst decorating at home. Twenty-four hours later the thumb became red, swollen and painful and he felt generally unwell. He was admitted to hospital under the care of the general physicians where he was found to be febrile and obviously unwell but quite indifferent to the state of his arm or his general well being. The right hand and forearm were diffusely red and swollen, without any clear cut margins (Fig. 1). There were several dusky, purple areas on the dorsum of the right hand and thumb and a large blister on the right thenar eminence. There was no lymphangitis or lymphadenopathy. He was treated with intravenous fucidin and erythromycin. Twenty-four hours later he was still obviously unwell, there were further dusky areas on the dorsum of his hand and he had developed thrombocytopenia (platelet count 50×10^9/l) and acute renal failure (blood urea 36·9 mmol/l; creatinine 381 μmol/l). A diagnosis of necrotizing fasciitis was made clinically and surgical debridement carried out, removing all the necrotic tissue from the thumb pulp to the elbow. Within 12 h he was afebrile and sitting up in bed, eating, drinking and passing urine. Forty-eight hours later his platelet count, blood urea and creatinine levels were back to normal. Three weeks later a split skin graft was applied to the debrided area and the arm was completely healed in 2 months.

The second group (patients 9–13) presented with what appeared to be cellulitis or erysipelas which did not improve with standard doses of intravenous or intramuscular benzyl penicillin. After an interval of several weeks the skin became necrotic. All these patients had been

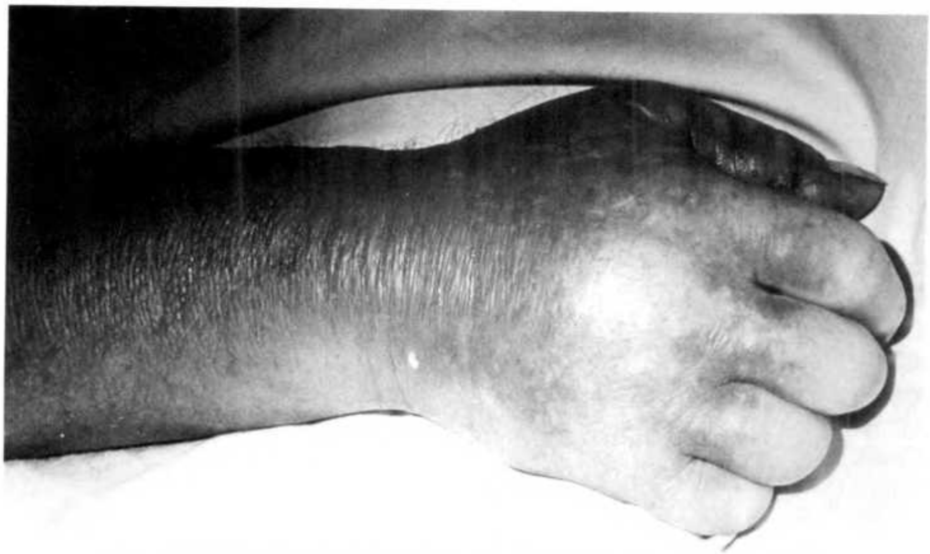

FIGURE 1. Necrotizing fasciitis. Appearance of hand on admission (patient 1).

previously fit, none was diabetic or receiving systemic steroids. All had received one or several courses of antibiotics before admission to hospital.

Case history—patient 10. A 68-year-old lady had been unwell at home for 2 weeks with a swollen right leg and foot, fever and general malaise. She gave no history of any injury to the foot or leg. She had been treated with oral phenoxymethylpenicillin by her general practitioner without any improvement. On admission to hospital, a clinical diagnosis of erysipelas was made because multiple blisters were present over the lateral aspect of the foot. Cultures from the blister fluid grew only *Streptococcus faecalis* and *Proteus sp.* She was treated with benzyl penicillin, 4 megaunits daily by intramuscular injection. Seven days later there was no sign of improvement and the blisters on her foot began to break down and discharge necrotic material. A diagnosis of necrotizing fasciitis was then considered. Three elliptical incisions were made over the dorsilateral aspect of the left foot and all the necrotic tissue down to and including the deep fascia was scraped out. Spontaneous healing occurred over the next 5 weeks.

The average length of hospital stay for this group of patients was 21 days after surgical intervention.

The third group consisted of a single patient who had a definite clinical diagnosis of necrotizing fasciitis, but no bacteriological or serological evidence of a streptococcal infection.

Case history— patient 14. A 79-year-old lady, on a small dose of prednisone for long-standing rheumatoid arthritis, was accidentally kicked on the left calf. A large bruise developed and 3 days later blisters occurred over the area. These were probed by the district nurse. Three weeks later on admission to hospital, virtually the whole of the medial aspect of her left calf was necrotic with a 10 cm^2 black eschar in the centre (Fig. 2). A diagnosis of necrotizing fasciitis was made and the whole of the necrotic area excised. Four weeks later, the debrided area was grafted by the plastic surgeons and it then completely healed after a further 6 weeks.

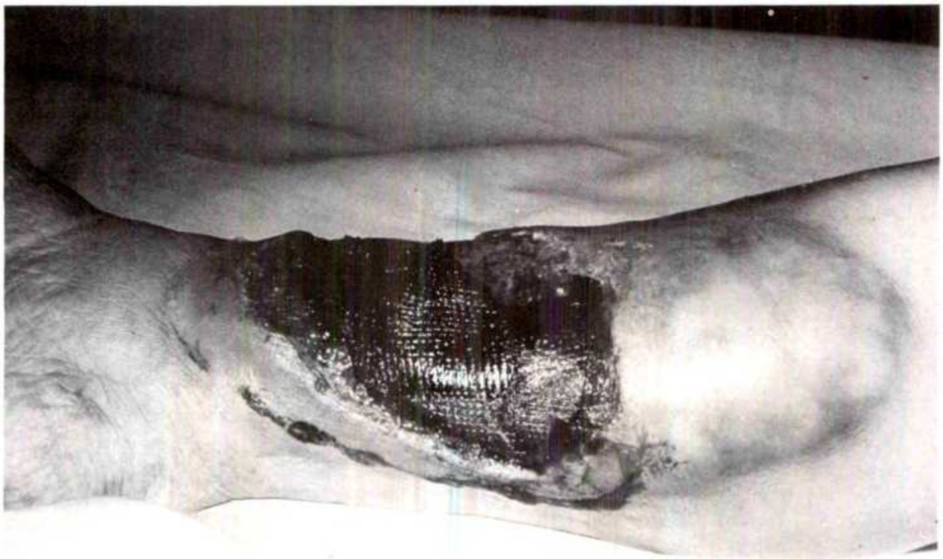

FIGURE 2. Necrotizing fasciitis. Leg of patient 14.

Bacteriology

A pure growth of *Streptococcus pyogenes* was grown in every case from the blister fluid and necrotic debris both on admission and from the surgical specimens of every patient who was acutely ill and who had not been treated with antibiotics before admission (patients 1–8). Different M types of *Streptococcus pyogenes* were found and both throat strains (patients 1–3) and skin strains (patients 4–8) occurred. Blood cultures were positive only in the man who died (patient 6).

In the five patients with a less severe clinical illness, all of whom had had one or more courses of antibiotics at home before admission (patients 9–13), bacteriological cultures were unhelpful; *Streptococcus pyogenes* was not isolated from any patient.

In the last individual (patient 14), a pure growth of *Staphylococcus aureus* was isolated.

Anaerobic organisms were not isolated from any patient, despite culturing for them on each occasion.

Serology

There was serological evidence of infection with *Streptococcus pyogenes* in twelve of the fourteen patients (numbers 1–5, 7–13) with markedly raised levels of ADB and AHT on admission. There was no obvious rise in titre over the first 2 weeks and in most patients the abnormal levels had returned to normal by 3 months. Normal levels only occurred in the patient who died within the first 24 hours in hospital. These tests were particularly helpful in patients with a less acute illness where *Streptococcus pyogenes* was not isolated. We cultured a Group G BHS from the debrided tissue of patient 12, but serologically she had a Group A, and not a Group G, BHS infection (Table 1). The ASO titre was normal or only slightly raised in most patients.

Blood counts and ESR

The white blood count and ESR were usually raised. Neither test was helpful in making the diagnosis since similar levels are found in erysipelas and cellulitis.

Histopathology

In eight patients, the debrided tissue was examined histologically (patients 1,2,5,7, 10–12, 14). All showed similar changes. The most striking feature in all the specimens was thrombosis of all blood vessels from the subcutaneous fat to the dermal papillae (Fig. 3) together with necrosis of the epidermis, dermis and subcutaneous fat. In some specimens, masses of polymorphonuclear leukocytes extended through the whole depth of the skin. In others they were most marked in the fascia, subcutaneous fat and throughout the walls of thrombosed vessels (Fig. 4). With routine Gram stains, Gram-positive cocci were not seen, but with the concentrated ammonium oxalate technique, masses of Gram-positive cocci were identified in and around the thrombosed vessels.

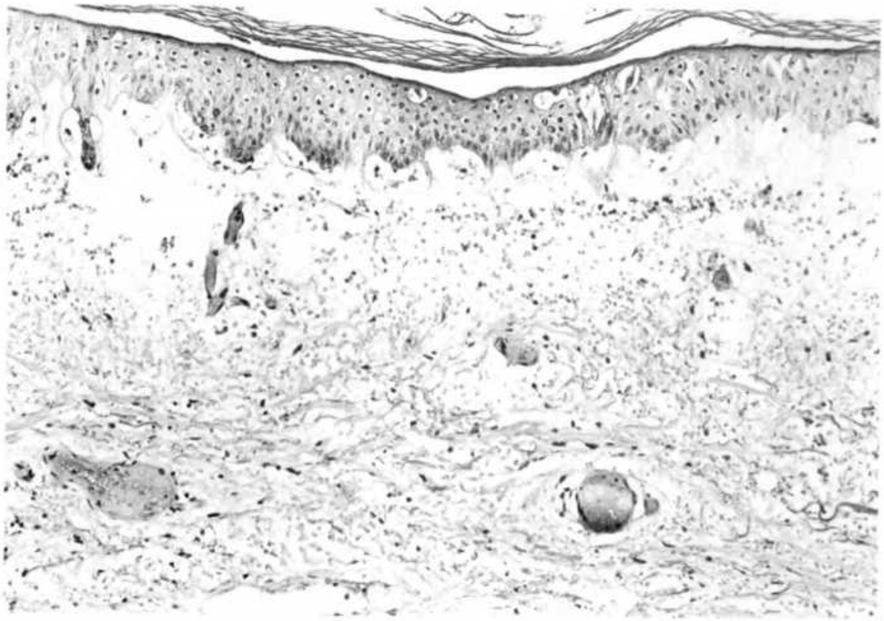

FIGURE 3. Histopathology from patient 1 showing necrosis of epidermis and dermis and thrombosis of blood vessels (H & E, × 90).

DISCUSSION

More than 50 years have passed since Lancefield (1928) identified the Group A streptococcus by serological grouping, and the organism is always sensitive to benzyl penicillin. Nevertheless there remain a group of patients who may have an acute fulminating illness due to this organism that can progress rapidly to death in spite of adequate antibiotic treatment. Individuals with the acute form of necrotizing fasciitis still need urgent surgical intervention for survival as was demonstrated in the pre-antibiotic era by Meleney (1924, 1933), and shown, for example, by

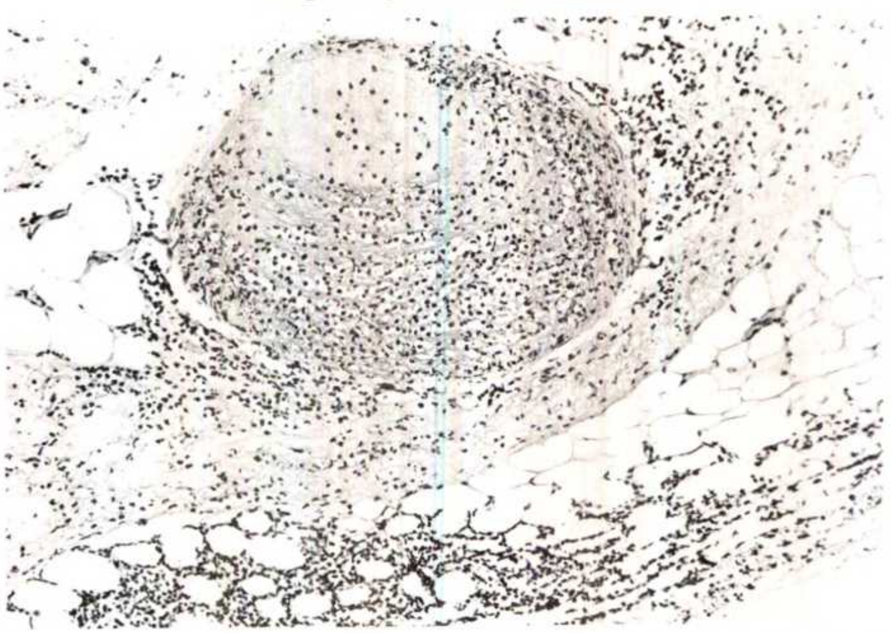

FIGURE 4. Histopathology from patient 1. Thrombosed vessel in subcutaneous fat infiltrated by polymorphonuclear leukocytes (H & E, × 122).

patient 1. There is still a considerable mortality (8–30%) from this disease (Wilson, 1952). This is partly dependent on associated illnesses, particularly diabetes (Crosthwait, Crosthwait & Jordan, 1964), but mainly on the time of diagnosis and delay in surgical intervention (Rea & Wyrick, 1970).

The essential feature of the disease is necrosis of the deep fascia and spread along the fascial plane with secondary gangrene of the overlying skin due to thrombosis of the blood vessels. Once this has occurred, bacteria can multiply within the necrotic tissue and are not usually eradicated by antibiotics, however high the dose. It seems likely that a bacterial necrotoxin is responsible for the pathogenesis of the condition.

In the acute illness, before the patient has received antibiotics, a pure growth of *Streptococcus pyogenes* can be isolated from blister fluid and the necrotic tissues. In most patients, cultures are best taken from the advancing edge of the lesion or from the central haemorrhagic bullae (Buchanan & Haserick, 1970). Occasionally an indistinguishable illness may be due to *Staphylococcus aureus*.

In the less acute form of the disease, and where the patient has been taking antibiotics, *Staphylococcus aureus* and other bacteria may colonize the wound, so that the causative organism, *Streptococcus pyogenes*, may not be recovered. It is in these patients that serological evidence of streptococcocal infection is most helpful in confirming the diagnosis. We have found that ADB is most consistently raised when necrotizing fasciitis is due to *Streptococcus pyogenes*, whilst the AHT can also be raised; both these enzyme preparations are specific for Group A BHS. This was particularly helpful in confirming the diagnosis in patient 12 from whom we cultured a Group G BHS. The ASO titre is not a useful test when the skin is the primary site of infection (Wannamaker, 1970), possibly because the antigenic effects of streptolysin O are inhibited by lipids which are abundant in the skin (Kaplan & Wannamaker, 1973). The measurement of ADB and AHT would also be useful in distinguishing necrotizing fasciitis from Meleney's post-operative progressive synergistic gangrene (Brewer & Meleney, 1926) and gas gangrene due to *Cl. perfringens* (welchii) with which the diagnosis can be confused (MacLennan, 1943).

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Pharmacology and Treatment

Beneficial effect of 15% azelaic acid cream on acne vulgaris

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SUMMARY

Patients treated with azelaic acid (15%) cream for chloasma reported simultaneous improvement of acne lesions within the treated areas. This prompted an open study of its effect in cases of acne without chloasma. One hundred patients with acne vulgaris were treated for 3–9 months by twice-daily application of the cream with significant improvement in every case.

Azelaic acid is a naturally-occurring straight-chained saturated dicarboxylic acid (1, 7-heptane-dicarboxylic acid). It is a competitive inhibitor of tyrosinase (Nazzaro-Porro *et al.*, 1979a) and has been shown to be effective in the treatment of hyperpigmentary disorders such as chloasma and lentigo maligna, and to have a cytotoxic effect on the human malignant melanocyte (Nazzaro-Porro *et al.*, 1979b,1980). While treating cases of chloasma with topical application of an azelaic acid cream, it was observed, and confirmed by patients, that lesions of acne within the areas being treated showed significant improvement. We decided to conduct a preliminary study to determine if cases of acne without chloasma, similarly treated, would benefit.

Acute, subacute, and chronic toxicity tests and tests for teratogenicity on rats and rabbits have established that azelaic acid is non-toxic (Mingrone *et al.*, 1983). No untoward reactions or side-effects of topical application for pigmentary disorders have been observed over a period of 4 years, and up to 20 gm a day have been taken orally by humans for periods of 6 months without ill effect. Its sodium salt (15%) has been administered intravenously, intra-arterially, and intra-lymphatically by continuous infusion for up to a week, without deleterious local or systemic effects.

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METHODS

One hundred unselected patients, ranging in age between 14 and 38 years were treated on a named patient basis for 3–9 months. The group included all types of acne of varying severity: comedonic, papulo-pustular, nodular, and several cases of acne conglobata. Patients were given a 15% azelaic acid cream (Nazzaro-Porro *et al.*, 1979b) and instructed to apply it, well rubbed in, to the affected areas initially once a day (first week or so), and subsequently twice a day. Results were monitored clinically and photographically, at first weekly, and then at fortnightly and monthly intervals.

RESULTS

During the first week, most patients reported a mild inflammatory reaction which was temporary. Within 1 to 2 months significant improvement was observed in all cases. Comedones, papules and pustules were less numerous, and the skin appeared less greasy. Some patients reported dryness of the skin. Nodules and cysts in severe forms of acne became softer, and either discharged or were resorbed. As treatment progressed, the improvement was maintained, and although new lesions appeared within the areas treated they were predominantly more superficial and in one patient's words, 'the cream seems to draw my spots out, and then they go away.'

Towards the end of treatment, many patients were applying the cream only to individual lesions as they appeared, and the situation could be kept under control by this means. Failure to apply cream regularly over a long period led often to relapse, which improved again with

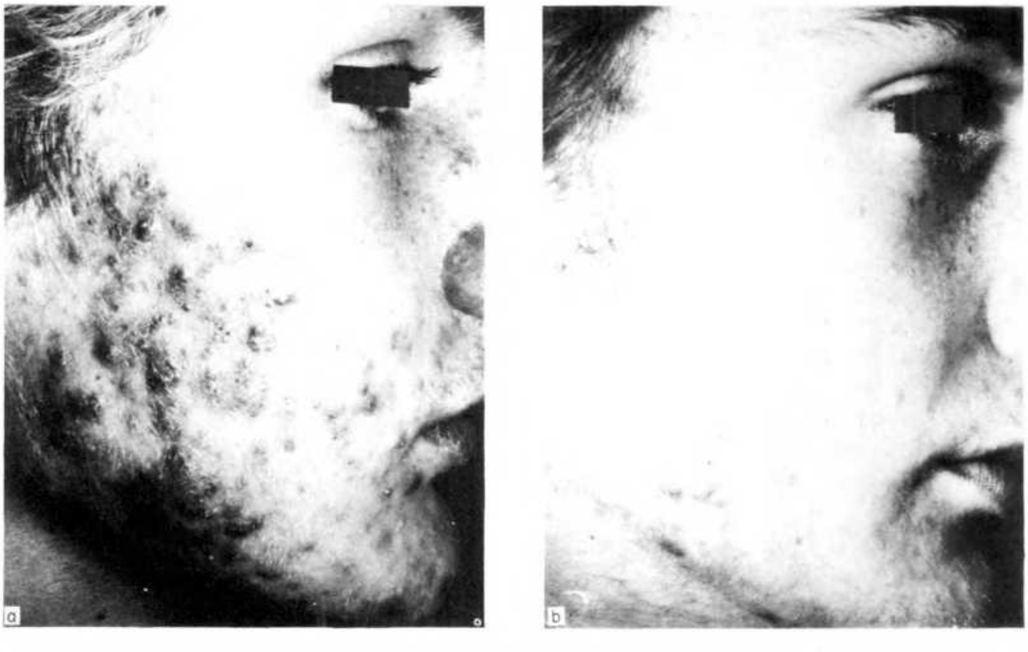

FIGURE 1. Nodular cystic acne of 2 years' duration in a boy aged 17. (a) Condition before treatment with azelaic acid cream, (b) after treatment for 10 months.

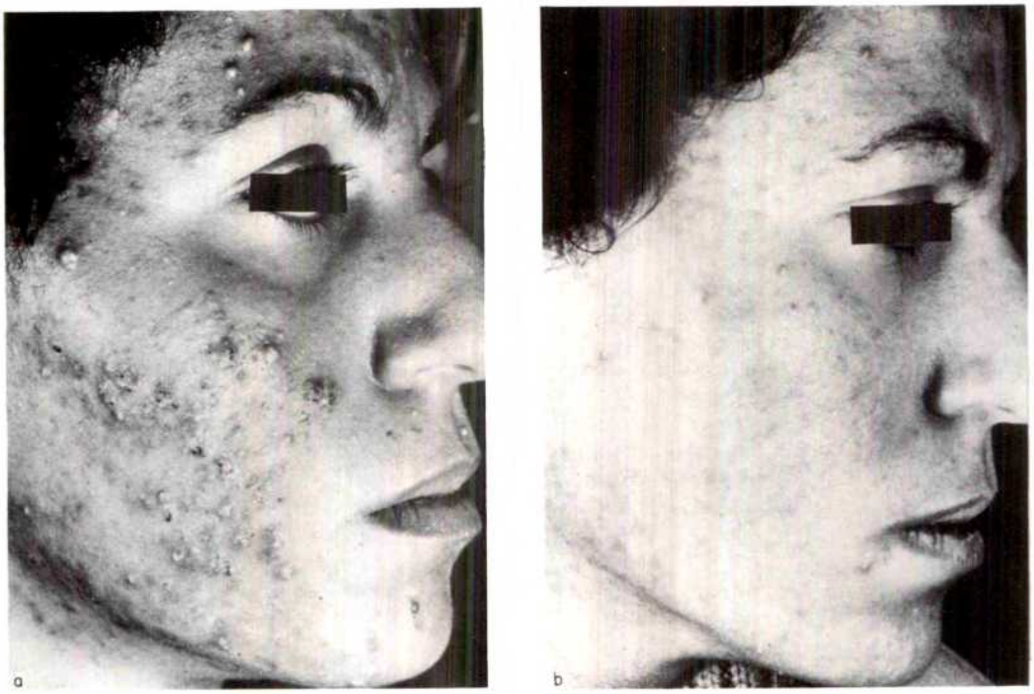

FIGURE 2. Pustular and nodular acne of 6 years' duration in a girl aged 18. (a) Condition before treatment with azelaic acid cream, (b) after treatment for 7 months.

re-application. Raised red relics of previous nodular lesions resolved and became less prominent as treatment progressed. Figs 1 and 2 show typical cases before and after treatment for 10 and 7 months, respectively.

DISCUSSION

Our results clearly indicate that azelaic acid, applied topically in a cream, has a significant effect on acne vulgaris. In our opinion, controlled clinical trials should now be performed to establish its clinical value as therapy in comparison with other current modes of treatment. At the moment, the duration of treatment must be regarded as long-term, although it can be interrupted once complete healing has been achieved.

It is not yet clear why azelaic acid should have a beneficial effect on acne as well as on hyperpigmentary disorders. Preliminary observations indicate that it is a competitive inhibitor not only of tyrosinase, but also of other oxido-reductases like some dehydrogenases of the respiratory chain (Picardo, Passi & Nazzaro-Porro, 1982). In addition we have established that in culture it exerts a bacteriostatic effect on aerobic and anaerobic bacteria, including *Propionibacterium acnes*. We have also observed, using hamster sebaceous gland, that *in vitro* it has a competitive inhibitory effect on the conversion of testosterone by 5-α-reductase to 5-α-dehydrotestosterone. Whether or not it affects sebum excretion rate or composition is being investigated.

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Treatment of cutaneous lymphoma with etretinate

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SUMMARY

Twelve patients with various types of lymphoma were treated with etretinate. The diagnosis included parapsoriasis en plaque, epidermotropic lymphoma (diffuse chronic erythroderma with atypical mononuclear cells, Sézary syndrome or MF tumours) and non-epidermotropic lymphoma. The patients received etretinate in a dose of 0·8 to 1·0 mg/kg/day for 2 to 14 months. No additional therapy was given. Patients with epidermotropic lymphomas stage I and II had a favourable clinical and histological response whereas those with deeply infiltrating tumours remained unresponsive. Patients with parapsoriasis en plaque and poikiloderma showed little response. Of the four patients who discontinued the treatment, three had recurrences after 3 to 4 months but one remained clear. The results obtained with etretinate may equal those obtained with more aggressive treatments.

Etretinate has been used to treat cutaneous T-cell lymphomas (Claudy, Delomier & Hermier, 1982a; Ippolito & Giacalone, 1982). We have now treated twelve patients with parapsoriasis en plaque and various stages of epidermotropic and non-epidermotropic cutaneous lymphomas (NECL) with this drug. This report concerns the clinical, histological and immunological effects of treatment.

PATIENTS AND METHODS

Twelve patients (seven men and five women, 47 to 77 years of age) with various types of cutaneous lymphomas have been studied. Clinical staging was based on the extent of skin involvement, the morphology of the lesions and the presence of extracutaneous disease as follows.

(a) *The parapsoriasis group* consisted of four patients who had either large plaques (three cases) or diffuse atrophic parapsoriasis (one case).

(b) *The epidermotropic lymphoma group* consisted of six patients with infiltrative erythroderma

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(three cases), Sézary syndrome (two cases), mycosis fungoides (MF) (one case). The stagir as follows (Abel *et al.*, 1981).

Stage I: epidermotropic lymphoma limited to the skin with no cutaneous tumours or ι *stage IA*: eczematous or limited plaque disease with involvement of 25% or less of the tota surface; *stage IB*: involvement of 25% or more of the total skin surface (included gener plaques and erythroderma).

Stage II: presence of skin tumours or histologically documented dermatopathic lympha pathy.

Stage III: epidermotropic lymphoma involving the skin with biopsy-documented lyn matous involvement of the lymph nodes or spleen.

Stage IV: cutaneous and extracutaneous epidermotropic lymphoma with docum visceral involvement.

(c) *The non-epidermotropic cutaneous lymphoma group* (NECL) (two cases). The patients low grade malignant B-cell lymphoma (lymphocytic type) and presented with mu non-ulcerated nodules and plaques.

The staging procedures included physical examination, chest X-ray, bone marrow asp liver and spleen scans, skin and lymph node biopsies (when clinically indicated), immunological evaluation with delayed skin tests using seven different bacterial and fi antigens, full blood count with differential, blood smears for Sézary cells, circulating percentage of sheep erythrocyte rosette-forming cells (E-RFC) and ratios of circul helper/suppressor cells using Orthoclone® monoclonal antibodies of different specifi (OKT3, OKT4, OKT8) (Ortho Pharmaceutical Corp., Raritan, NJ), lipoprotein electroj esis and auto-antibody screen. None of our patients had staging laparotomy.

The tests were done prior to treatment and after intervals of 1 and 3 months. The durati overt lymphoma from diagnostic biopsy to treatment ranged from 1 to 18 months. S patients had failed to respond to previous treatments such as topical corticosteroids, tc mechlorethamine, PUVA or systemic chemotherapy (Table I).

The patients received etretinate (RO 10 9359) in a dose of 0·8 to 1·0 mg/kg/day, infoı consent having been obtained in all cases. Duration of therapy varied from 2 to 14 month additional topical or systemic therapy was given. The maintenance regimen employed in patients was either 1 mg/kg/day or 0·5 mg/kg/day, depending on the clinical response intensity of the side-effects, if any, and the serum lipid profiles.

Biopsies were taken from the most advanced lesions and repeated at areas adjacent to the biopsy from sites with maximum skin involvement or from previously involved sites clinical clearing.

RESULTS

Clinical evaluation (Table 2)

In the four patients with parapsoriasis en plaque or diffuse atrophic parapsoriasis, whose di had been evolving for many years, the clinical result was poor according to the patients' anc own assessment.

In the three patients with generalized erythroderma stage IB, the results were impres Within 2 to 4 weeks, the redness faded, the infiltration almost disappeared, palms and soles their hyperkeratotic appearance, enlarged lymph nodes became smaller or were no lo palpable and pruritus stopped.

TABLE 1. Patients treated with etretinate

Patient No.	Age	Sex	Duration of disease†	Stage of disease	Previous therapy
1	55	M	45 mo.	Parapsoriasis en plaque	Topical corticosteroids
2	64	M	22 yrs	Parapsoriasis en plaque	Topical corticosteroids
3	69	F	27 yrs	Parapsoriasis en plaque	Topical corticosteroids
4	47	M	9 yrs	Atrophic parapsoriasis	PUVA, topical mechlorethamine
5	55	M	16 mo.	IB	—
6	72	M	1 mo.	IB	—
7	75	M	7 mo.	IB	Topical corticosteroids
8*	77	F	2 mo.	II	—
9	72	F	18 mo.	III (Sézary)	Chemotherapy, corticosteroids
10	71	F	6 yrs	III (MF tumours)	PUVA
11	61	F	1 mo.	NECL	—
12	69	M	6 mo.	NECL	—

* Case published in *Archives for Dermatological Research*, **273**, 37.

† Histologically proven.

NECL = non-epidermotropic cutaneous lymphoma.

TABLE 2. Results of etretinate therapy

Patient No.	Stage of disease	Duration of treatment (months)	Clinical state	Histological state
1	PP	5	Unchanged	Slightly improved*
2	PP	4	Slightly improved	Slightly improved
3	PP	3	Unchanged	Slightly improved
4	AP	2	Unchanged	Slightly improved
5	IB	7	Clear	Markedly improved†
6	IB	14	Clear	Markedly improved
7	IB	5	Clear	Markedly improved
8	II	4	Clear	Markedly improved
9	III	8·5	Clear	Markedly improved
10	III	2·5	Worsening	Unchanged
11	NECL	6	Markedly improved	Improved
12	NECL	3	Unchanged	Improved

* Epidermis cleared, dermal infiltrate unmodified.

† Epidermis cleared, density of the dermal infiltrate greatly diminished.

PP = parapsoriasis en plaque; AP = atrophic parapsoriasis; NECL = non-epidermotropic cutaneous lymphoma.

In patient 8, as we have already reported, small multiple skin tumours disappeared but recurred after stopping the drug (Claudy *et al.*, 1982a).

In patient 9, who had typical Sézary syndrome, progress seemed moderately encouraging after 3 months, but marked hyperkeratosis and pruritus persisted. However, after 8-months' treatment, at a dosage of 1 mg/kg/day, the erythroderma was successfully controlled (Figs 1 and 2), and the superficial lymph nodes were no longer palpable.

Patient 10, with large ulcerated tumors, got worse during the 10-week treatment period, and the drug had to be discontinued.

Of the two patients with NECL, one responded well and achieved relative clearing within the 3-month trial period; the other remained unchanged, but the follow-up period was too short to assess the long-term outcome.

Among the four patients who discontinued the treatment after clinical clearing, three had recurrences after 3 to 4 months.

Histological evaluation (Table 2)

In the parapsoriasis group, the upper dermal and epidermal infiltrates were mild and the changes after treatment were not striking.

In the epidermotropic lymphoma group, the specimens of the three stage IB patients and of the stage II patient, who had numerous dense hyperchromatic mononuclear cells with convoluted nuclei infiltrating the upper dermis and epidermis (Pautrier microabscesses) showed, after treatment, a disappearance of the epidermal infiltrate and a marked reduction of the dermal infiltrate which was separated from the dermo-epidermal junction by a thin band of normal collagen (Fig. 3). Despite the good clinical response, complete histological clearing was never obtained. In case 9 (stage III), the epidermal infiltrate of mononuclear cells cleared, but a patchy infiltrate around vessels in the upper dermis remained. In case 10 (MF tumours), no changes occured in the dermal infiltrate, but the intra-epidermal mononuclear cell aggregates were no longer present.

In the NECL group, a marked reduction of the dermal infiltrate was noted, but no complete clearing.

Immunological evaluation (Table 3)

The delayed skin test responses to seven common bacterial and fungal antigens were within normal limits in eight of ten patients before treatment and were not done after treatment. The absolute number of circulating lymphocytes remained unchanged. In the Sézary syndrome (case 9), the number of circulating atypical cells fell from $1400/mm^3$ to $400/mm^3$, but this may have been purely coincidental. IgE levels were elevated in cases 5 and 7, although there was no history of atopy. E-RFC evaluations were within normal limits (except in cases 5 and 10) before treatment and the alterations noted after treatment were thought not to be significant.

The OKT ratios (helper/suppressor cells) were normal in the parapsoriasis and the NECL groups, and were markedly elevated in all the epidermotropic lymphoma patients. No changes were observed after the 3-month treatment period.

Side-effects

Almost all patients experienced a mild cheilitis and a few of them had dryness of the nasal mucosa and xerosis. No severe toxic effects were seen even at a dosage of 1 mg/kg/day.

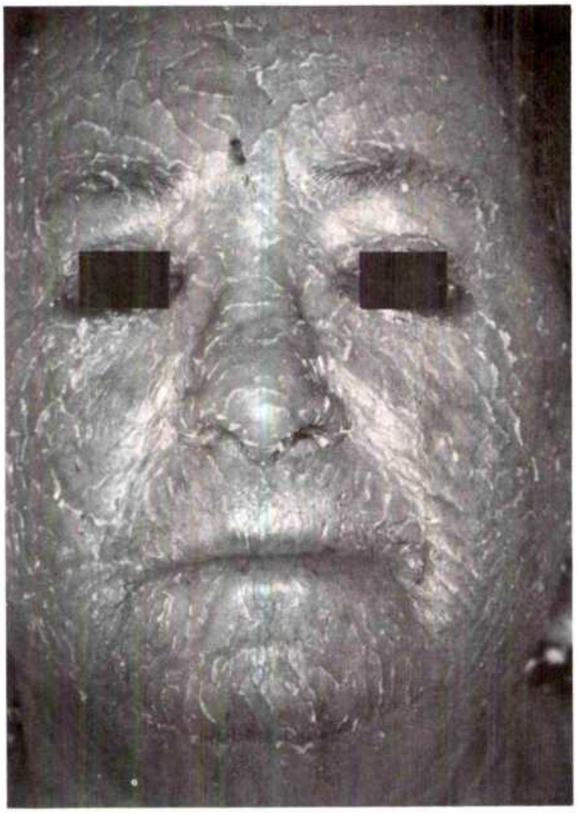

FIGURE 1. Patient 9. Clinical appearance before treatment.

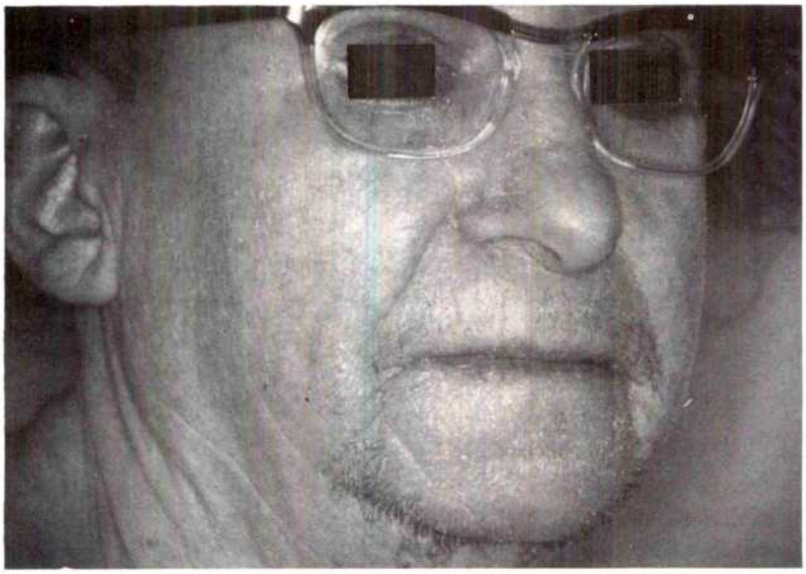

FIGURE 2. Patient 9. Clinical appearance after treatment.

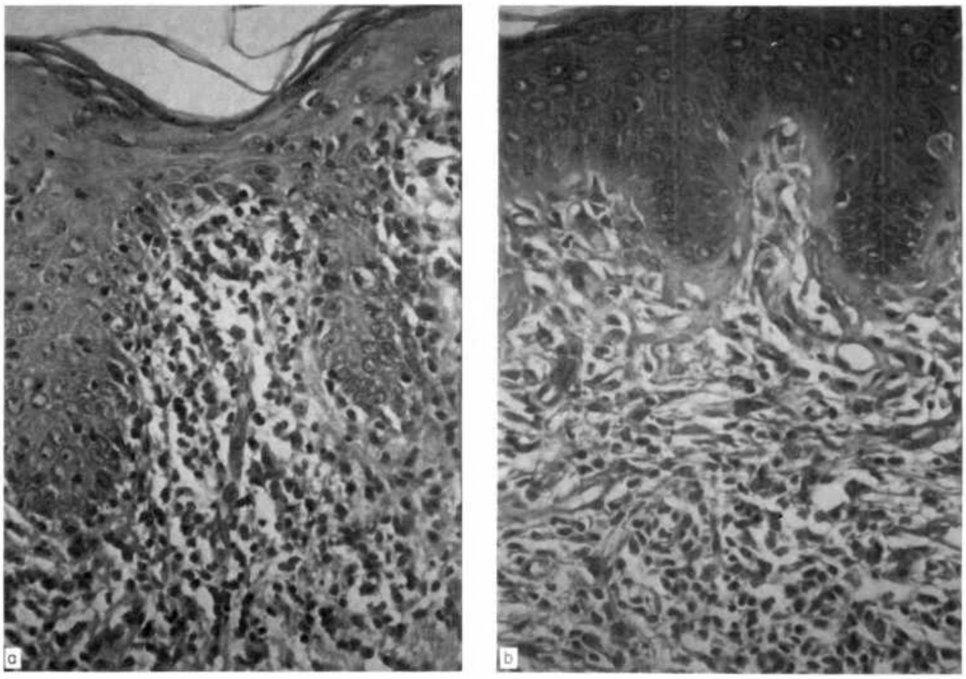

FIGURE 3. Histological appearance of epidermotropic lymphoma (a) before treatment and (b) after treatment. Note the absence of intra-epidermal atypical mononuclear cell infiltrate and the presence of a band-like spared subepidermal area in the papillary dermis after treatment (H & E, × 200).

TABLE 3. Immune status

Patient No.	Tests of delayed hypersensitivity	Lymphocyte/mm³		Sèzary cells (> 10%)	IgE iu/ml	E-RFC (%)		H/S	
		Before	After			Before	After	Before	After
1	10·5*/2**	2000	2700	–	27	77	80	1·4	1·5
2	9·5/2	2200	2400	–	5	67	ND	1·9	ND
3	ND	ND	2024	ND	11	ND	82	ND	7·6
4	23/3	1500	2000	–	7	71	ND	1·4	1·3
5	6/1	1200	1400	–	320	52	70	3·9	3·5
6	34/5	1600	1800	+	ND	76	69	3·8	4·8
7	0	940	820	–	520	55	73	2·9	3·4
8	ND	1620	1300	–	42	63	ND	ND	ND
9	0	9000	12000	+	105	72	77	17·7	9·4
10	8/2	2600	2100	+	150	56	60	4·4	3·8
11	24/4	1600	1600	–	5	65	ND	1·77	ND
12	11·5/3	2200	1920	–	43	84	70	1·08	ND
Controls					< 100	72 ± 8		1·77 ± 0·54	

* Sum of mean diameters (mm) of all positive reactions observed at 48 h with the following antigens: tuberculin, tetanus and diphtheria anatoxins, candida, proteus, streptococcus, trichophyton.

** Number of positive skin reactions.

H/S = helper/suppressor = OKT4+/OKT8+ (OKT4+ = OKT4/OKT3 × 100; OKT8+ = OKT8/OKT3 × 100).

ND = not done.

No modifications in serum glutamic oxalacetic or glutamic pyruvic transaminase concentrations were noted. A two-fold increase in blood triglycerides was found in three of the nine patients who were examined before and during the treatment. These three patients were considered at risk because of previously existing obesity or diabetes. There was no relationship of the clinical side-effects to hypertriglyceridemia.

DISCUSSION

Several therapies have been used for cutaneous lymphomas, including PUVA, topical and systemic chemotherapy and electron beam therapy (Vonderheid, 1980). Most of these treatments have a beneficial effect at early stages of the disease but the effects are usually of short duration. Control studies have, as a rule, not been done and no treatment has yet been shown to prolong survival.

Our reason for trying aromatic retinoids in cutaneous lymphomas was that several animal studies and clinical trials suggested that tumour promotion may be suppressed by these drugs (Bollag, 1974; Bollag & Ott, 1975; Claudy *et al.*, 1982b). Our present results support the previously reported observations of the effect of etretinate in patients with cutaneous lymphomas confined to skin and superficial lymph nodes (Claudy *et al.*, 1982a). However, despite objective clinical improvement in skin lesions and disappearance of enlarged lymph nodes, the treatment appeared to be merely palliative and not curative, since dermal infiltrate never cleared completely. The clinical benefit was more apparent in patients with epidermotropic lymphomas at stage IB and II. In the parapsoriasis and NECL groups, the clinical improvement was only slight. One patient with MF tumours stage III deteriorated during the treatment. Four patients discontinued the treatment and three of them had recurrences in 3 to 4 months.

In all cases, slight or marked changes in the density of the mononuclear cell infiltrate were noted. When present, the intra-epidermal aggregates of atypical cells were eradicated and the dermal infiltrate became either patchy or band-like, and separated from the dermo-epidermal junction by a thin layer of normal collagen. In the NECL group, the reduction of the dermal infiltrate was also apparent.

Immunological measurements, such as the absolute number or circulating lymphocytes, the percentage of E-RFC or the ratios of helper/suppressor cells, as assessed by monoclonal antibodies, were not significantly different after the treatment.

The possible mechanisms of action of the retinoids have been extensively reviewed (Elias & William, 1981; Dicken & Connolly, 1982). They include immunomodulation, anti-inflammatory effects with steroid hormone-like action, suppression of ornithine decarboxylase, lysosomal labilization, alteration of cell membrane functions, and post-translational glycosylation. In patients with cutaneous lymphomas, retinoids may play major roles as an adjuvant for induction of cytotoxic cells and as a regulator of the interaction between T lymphocytes and epidermis.

Epidermis is a milieu reminiscent of the thymus in which precursor lymphocytes may differentiate (Streilein, 1978; Schmitt, 1981). T cells which multiply to form a clone of malignant cells have a strong affinity for epidermis at early stages of cutaneous lymphoma. As the cells proliferate, they no longer exhibit affinity for epidermis and disseminate to lymph nodes, blood and viscera (Edelson, 1980). One may wonder whether the transformation of an epidermotropic infiltrate into a more aggressive non-epidermotropic infiltrate by the aromatic retinoid may not facilitate, over a medium or long-term period, the 'natural' evolution of cutaneous T-cell lymphomas, leading the malignant cells to lose their affinity for epidermis and permitting invasion of internal organs.

The clinical and histological short-term results obtained with etretinate in twelve patients with cutaneous lymphomas seemed to equal the results reported with alternative treatments which are usually more aggressive and not devoid of severe side-effects. Further studies will be needed to establish whether etretinate has an effect on survival of these patients.

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Dapsone in the treatment of miliary lupus of the face

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SUMMARY

In an uncontrolled study of twelve patients with miliary lupus of the face, dapsone gave good therapeutic results.

Miliary lupus of the face (MLF) is an asymptomatic papular eruption affecting the central area of the face, which runs a chronic course and involutes spontaneously with scarring. Histopathologically, the papules usually show scattered masses of tuberculoid granulomata composed of epithelioid cells, giant cells, and an encircling rim of small round mononuclear cells in the dermis. The aetiology and pathogenesis of this condition are still unknown. The treatment of MLF has not been satisfactory despite the use of many topical and systemic agents.

In this paper we report that dapsone has been found to be beneficial in the treatment of MLF and has accelerated its resolution in twelve patients.

CASE REPORTS

The details of twelve patients with MLF responding to systemic dapsone are summarized in Table 1. They consisted of eight males and four females, aged from 15 to 60 years. All patients showed the characteristic clinical manifestations of MLF, exhibiting multiple red or a few white to whitish-yellow papules 1 to 2 mm in diameter on the face. The papules were both follicular and non-follicular and were bilaterally distributed in the centrofacial regions. The eyelids were particularly involved.

Before dapsone treatment, biopsy specimens were obtained from all the patients. Each specimen showed a tuberculoid infiltrate in the dermis composed of epithelioid cells and a few giant cells surrounded by a round-cell infiltrate with or without central necrosis. In some cases, biopsies were performed again in the course of treatment in order to evaluate the action of dapsone. In all patients, a PPD skin test was positive, but neither the chest X-ray nor the past history revealed any evidence of tuberculosis. The duration of the disease before dapsone treatment varied from 2 to 50 months. Most of the patients had been previously treated with

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TABLE 1. Case summaries of the MLF patients treated with dapsone

Case	Age/Sex	Duration before treatment (months)	Dose of dapsone (mg/day)			Cure interval (months)
			Initial	Increased in slowly responding cases	Reduced after cure	
1	38/M	2	50		25	3·5
2	23/M	8	50		25	3·0
3	18/F	20	25	50		2·5
4	41/F	50	25			8·5
5	28/M	13	25	50		3·5
6	32/M	6	25	50	25	8
7	46/F	14	50			5
8	60/M	3	25	50	25	11
9	34/M	5	75			3·5
10	27/F	6	50			7·5
11	29/M	12	50	75		8·5
12	15/M	2	50			4·5

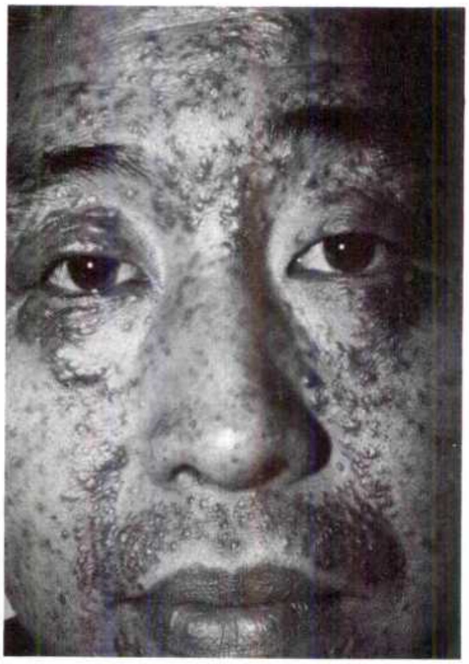

FIGURE 1. Miliary lupus of the face. Discrete red papules, 1–2 mm in diameter, bilaterally localized on the forehead, eyelids, cheeks and chin (case 1).

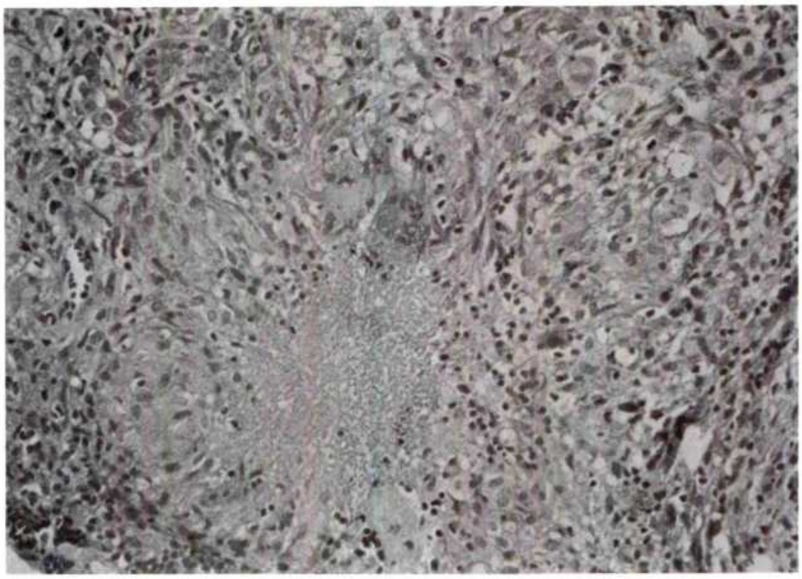

FIGURE 2. Biopsy of a periocular lesion showing tuberculoid infiltrate composed of epithelioid cells with central necrosis surrounded by round-cells.

steroids, tetracycline, and various anti-tuberculous agents topically and/or systemically with only temporary benefit. Three of the patients (Nos 8, 9 and 12, Table 1) had never received any drugs previously when dapsone treatment began.

In the majority of patients oral administration of dapsone was started with 50 mg/day. Most of the patients were found to be cured within 5 months of starting medication. Dapsone was

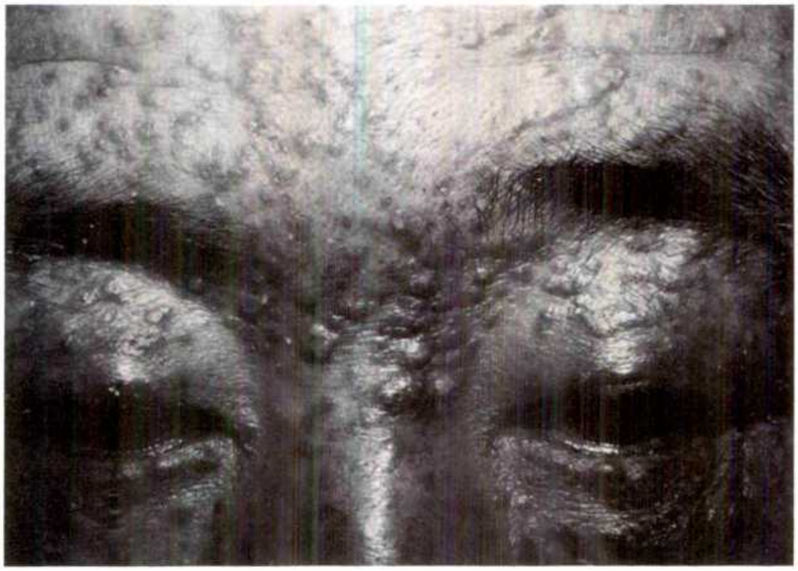

FIGURE 3. Two weeks after starting dapsone.

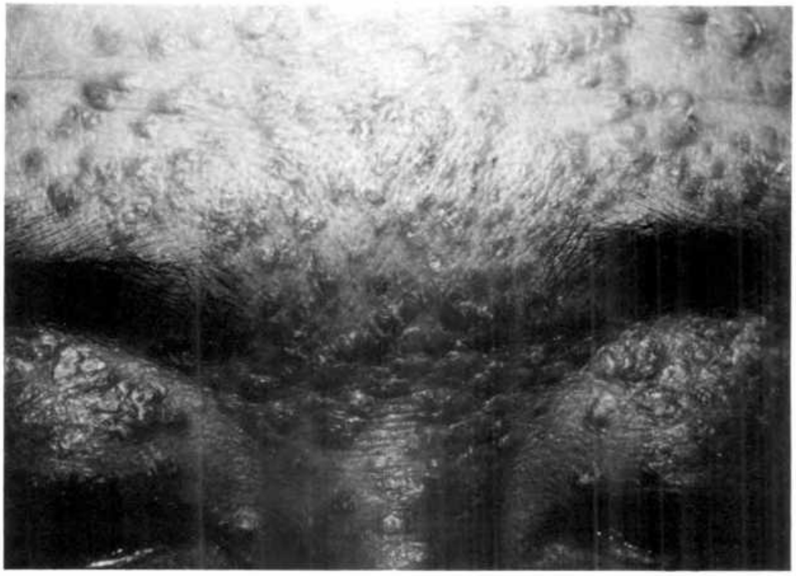

FIGURE 4. Six weeks after starting dapsone.

stopped 7 months after the initiation of medication. No undesirable side-effects of the drug were noticed during therapy. There have been no recurrences in the 7 to 24 months of follow-up.

An illustrative case

Case 1. A 35-year-old Japanese male attended our clinic in December 1979 with periocular asymptomatic red papules of 2 months' duration. He had been treated with oral steroids at another clinic without improvement of his condition. He had no history of tuberculosis. The following laboratory findings were within normal limits: blood sedimentation rate, complete blood count and chest X-ray. A PPD skin test was positive. Discrete red papules, 1–2 mm in diameter, were bilaterally distributed on the forehead, eyelids, nose, cheeks and chin (Fig. 1). Biopsy revealed tuberculoid histology (Fig. 2). Acid-fast organisms were not observed with Ziehl-Neelsen's stain.

In January 1980 oral dapsone, 50 mg daily, was initiated. One week later most of the red

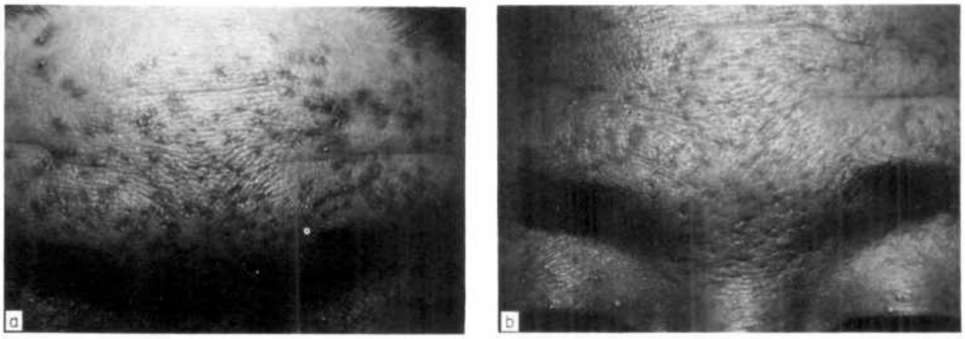

FIGURE 5. (a) After 16 weeks almost all active papules have subsided with many dark red depressed scars. (b) At 28 weeks only scarring remains.

papules became dark in colour and some of them had yellowish-white centres (Fig. 3). Two weeks later these papules with yellowish-white centres became the predominant lesions, some showing central crater-like pits. Gradually the papules were covered with pityriasiform scaling (Fig. 4). Almost all of the active papules subsided leaving small depressed scars, 10 to 12 weeks after starting therapy (Fig. 5a). At this stage, the dosage of dapsone was reduced gradually, and discontinued 7 months after starting treatment (Fig. 5b).

DISCUSSION

The aetiology of MLF is unknown. There is no evidence supporting a link with tuberculosis. Non-tuberculous causes such as a papular type of rosacea and micropapular forms of sarcoidosis or sarcoidal reactions have been discussed (Lever & Lever, 1975; Simon, 1975; Ackerman, 1978; Fitzpatrick *et al.*, 1979; Rook, Wilkinson & Ebling, 1979). Many assumptions as to the cause and pathogenesis of this condition have been controversial.

A number of agents have been used to treat MLF. Many clinicians have reported that anti-tuberculous drugs such as isoniazid, streptomycin, para-aminosalicylic acid and rifampicin, some antibiotics, e.g. tetracycline (Simon, 1975), sporotrichin, transfer factor, and tuberculosis vaccination are effective. On the other hand the non-effectiveness of steroids, anti-tuberculous drugs, anti-malarial drugs, vitamin B_2 and B_6 has also been reported.

MLF is a chronic disease with spontaneous involution. In the evaluation of the therapeutic efficacy of dapsone, the natural course of MLF must be considered. It usually runs a course of 12 to 24 months before disappearing spontaneously with scarring (Lever & Lever, 1975; Ackerman, 1978; Fitzpatrick *et al.*, 1979; Rook *et al.*, 1979). However, as was seen in case 4 (Table 1) some appear to have a long course of more than 50 months.

In new cases of MLF such as 1 and 9 (Table 1), cure intervals for dapsone treatment were about 3·5 months. In these cases also, dapsone could be discontinued after 7 months of medication, i.e. dapsone appeared to alter the natural history and eventual resolution of MLF. Clinically, almost all papules in each case began to change from red to yellowish-white at about 2 weeks after starting treatment, and then developed crusts or pityriasiform scales and pits on their summits. They subsequently involuted leaving pitted atrophic scars. It was surprising that these changes occurred simultaneously in almost all papules of patients treated with dapsone while in untreated patients various stages of papules could be found. This also suggests that dapsone alters the natural history of MLF.

Although used in the treatment of many skin conditions, the mechanism of action of dapsone remains unclear (Lang, 1979). Pharmacologically dapsone acts as an inhibitor of folic acid synthesis in infectious organisms. It is also reported that dapsone inhibits the release of lysosomal enzymes (Barranco, 1974; Mier & Van den Hurk, 1975), chemotaxis of polymorphonuclear leukocytes, the process of conservation of sodium iodide to I_2 by neutrophils (Stendahl *et al.*, 1978) and PHA-induced lymphocyte transformation (Beiguelman & Pisani, 1974). It is also known that dapsone inhibits the alternate pathway of complement (Stendahl *et al.*, 1978) and its haemolytic effect has in part been attributed to its inhibitory effect on the transport of adenosine through the erythrocyte membrane (Van Dyke, 1972).

It has a general anti-inflammatory action (Williams *et al.*, 1976), and suppresses the Arthus reaction and enhances the action of corticosteroids due to its steroid-sparing effect when used concomitantly (Lang, 1979).

Although we do not know how dapsone works in MLF, we have reported here the efficacy of dapsone in this disease. A controlled trial of of dapsone in MLF as well as studies to elucidate its mode of action are now required.

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A double-blind study of 1% metronidazole cream versus systemic oxytetracycline therapy for rosacea

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SUMMARY

In a randomized double-blind trial fifty-one patients with rosacea were treated for 2 months with either 1% metronidazole cream and placebo tablets or with 250 mg oxytetracycline tablets taken twice daily, and placebo cream (the cream base). The patients were assessed before and at the end of the trial, using the following criteria: (1) overall clinical assessment, (2) lesion counts, (3) degree of erythema, (4) independent photographic evaluation, (5) patients' opinion. An improvement was shown in 90% of the patients of both groups, and there was no significant difference between the two treatments.

One per cent metronidazole cream has been shown to be significantly better than a placebo cream in the treatment of rosacea (Gamborg Nielsen, 1983a). It was therefore considered important to compare the cream with conventional therapy, and for this reason a double-blind study of 1% metronidazole cream versus a daily dose of 500 mg oxytetracycline was performed.

METHODS

Fifty-one randomly selected patients (thirty-four women and seventeen men, average age 44 years) with rosacea entered the trial, which took place during March, April and May of 1982. None of the patients had been treated with drugs active against rosacea during the 3 months preceding the start of the trial.

Patients were assigned at random to one of the two courses of treatment. Twenty-five were allocated to 1% metronidazole cream and placebo tablets and twenty-six to oxytetracycline tablets 250 mg twice daily, and placebo cream.

Patients were provided with 50 g of the test cream, which was applied once daily for 2 months. The cream base used for both the placebo and the metronidazole cream was an oil in water emulsion (lactic acid 1·5%, sodium lauryl sulphate 0·8%, cetylane 5%, cetanole 15% and

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distilled water 80%). Tablets were administered twice daily 30 min before meals. None of the patients were allowed to use any other treatment for their rosacea during the study period.

Before and at the end of the trial each patient underwent an overall clinical examination. including lesion counts (papules, pustules and telangiectases) and degree of erythema; this was combined with standardized photographic evaluation of the rash (Gamborg Nielsen, 1983b). At the end of the trial the patients' subjective opinion of the treatment was registered. Patients were examined for side-effects and special attention was paid to possible allergic or irritant reactions to the cream.

RESULTS

Twenty-five patients receiving 1% metronidazole cream, and twenty-three receiving oxytetracyclines completed the study. Two patients became pregnant during the study period (they stopped treatment and were excluded) and one left without reason. All three were from the oxytetracycline group. The results of the overall clinical assessment and photographic evaluation showed that there was no significant difference between the two courses of treatment (Table 1). Reduction of erythema, papules and pustules was the same in both groups, and the number and extent of telangiectases were unchanged. No side-effects were reported in either group.

TABLE 1. Number of improved patients in the metronidazole and oxytetracycline groups assessed by clinical estimation, photographic evaluation, and subjective opinion of treatment results

	1% Metronidazole cream	Oxytetracycline (500 mg)
Objective		
Clinical	24/25 (96%)	22/23 (96%)
Photographic	21/25 (84%)	21/23 (91%)
Subjective		
Patients' opinion	22/25 (88%)	21/23 (91%)

DISCUSSION

In several publications it has been shown that orally administered metronidazole is an effective treatment for rosacea (Pye & Burton, 1976; Schirner & Haneke, 1981), and also that response to such treatment is as good as that with oxytetracycline (Saihan & Burton, 1980). Recently we have shown that in rosacea topical application of metronidazole is significantly better than placebo. In the present study it was demonstrated that response to this cream does not differ significantly from that produced by systemic oxytetracycline. It has been shown that transcutaneous absorption from 1% metronidazole cream results in a blood level that is at most 1% of the level reached when the minimum oral dose necessary for improvement of rosacea is administered (Arnold, 1982). Thus fewer systemic side-effects would be expected and we found none.

ACKNOWLEDGMENTS

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Photochemotherapy (PUVA) in the treatment of urticaria pigmentosa

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SUMMARY

Eight patients received PUVA for mastocytosis. Five women had typical adult-onset urticaria pigmentosa, without evidence of systemic disease. Another woman had suspected hepatic involvement while the remaining female had early-onset familial urticaria pigmentosa with morphologically atypical mast cells. The only male patient had cirrhosis with hepatic deposits of mast cells in addition to polycythaemia rubra vera. In all patients, except the man with systemic disease, there was reduced pruritus and wealing and partial to almost complete fading of the macules. The manifestations of urticaria pigmentosa recurred after treatment was discontinued. In both lesional and uninvolved skin there was no significant change in either the mean mast cell counts or mast cell ultrastructure after an average of twenty-seven PUVA exposures. In addition, PUVA did not cause a significant alteration in the histamine content of the skin.

The beneficial effect of PUVA in urticaria pigmentosa therefore does not appear to be directly related to a change in mast cell numbers or morphology, or to the histamine concentration in the skin.

Urticaria pigmentosa usually presents as a generalized maculo-papular rash which urticates on rubbing (Darier's sign). Many patients are troubled only by the unsightliness of the rash while some complain of pruritus, wealing or flushing. These symptoms are attributed to the release of histamine by mast cells which characteristically occur in increased numbers in the dermis.

Symptomatic treatment is often unrewarding, but favourable results have been claimed for cimetidine with or without H_1 blockers (Hirschowitz & Groarke, 1979; O'Laughlin &

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Bredfeldt, 1980) and for oral disodium cromoglycate (Soter, Austen & Wasserman, 1979; Czarnetski & Behrendt, 1981). In 1978, Christophers and colleagues reported that photochemotherapy (PUVA) produced symptomatic relief in all of ten adult patients with typical urticaria pigmentosa. Similarly encouraging results were subsequently reported from other centres (Ortonne *et al.*, 1980; Allevato, Donatti & Cordero, 1980; Granerus, Roupe & Swanbeck, 1981; Väätäinen, Hannuksela & Karvonen, 1981).

In this study we examined the effects of PUVA in eight adult patients with urticaria pigmentosa. Although PUVA produced a moderately good clinical response in seven out of these eight patients (reduced pruritus, reduced wealing and faded macules) quantitative studies failed to reveal a consistent effect of PUVA on either the mast cell population density or the histamine concentration in both lesional and clinically uninvolved skin. The findings are discussed in relation to existing information concerning the effect of ultraviolet radiation (UVR) on mast cells and other constituents of the skin.

METHODS

Eight adult patients were studied (Table 1). Patients T.K. (R18706), Y.W. (P89642), T.L. (R25970), P.A. (CA0200) and A.K. (AH8040) had typical adult-onset urticaria pigmentosa, and routine laboratory investigations were normal in them. These tests included full blood count, erythrocyte sedimentation rate, serum urea and electrolytes, liver function tests, serum protein electrophoresis, urinalysis and chest X-ray.

Patient L.C. (R18412) had an eosinophilia (12% out of a total of 6000 white blood cells), her serum IgE was raised to 940 iu/ml (normal = 50–500 iu/ml) and the liver and spleen scan revealed a filling defect at the upper posterior pole of the left lobe of the liver in addition to an inactive mass extending across the base of the organ.

A detailed report on patient D.P. (R11980) has been published (James & Eady, 1981). The rash had several unusual clinical and biological features. It was present since infancy, and her elder sister was affected by the same disease, although to a lesser extent. There were numerous pigmented macules, papules and nodules mainly on the trunk, associated with intense pruritus. Rubbing, warm baths and alcohol ingestion caused wealing and flushing. The clinically

TABLE 1. Details of patients and PUVA regimen

					PUVA between biopsies			
Pt.	Centre*	Sex	Age	Duration of disease (Years)	Initial dose UV-A (J/cm²)	Exposures	Total dose UV-A (J/cm²)	UV-A dose per exposure at biopsy (J/cm²)
T.K.	L	F	24	10	1·5	22	78·5	9
L.C.	L	F	44	6	2·5	20	64	4
Y.W.	L	F	34	10	1·5	69	396·5	6·5
T.L.	L	F	46	12	1·5	23	66·5	5
D.P.	L	F	20	20	1·5	16	20·5	2
R.W.	L	M	58	2	1·5	28	228	12
P.A.	S	F	40	6	2	15	50	4
A.K.	S	F	86	12	1·5	24	89	9

* L = London, S = Sheffield

uninvolved skin was dermographic. Routine biochemical and haematological investigations, bone marrow aspirate, liver and spleen scan and skeletal survey were normal. Lesional and non-lesional mast cells contained giant cytoplasmic granules with an abnormal ultrastructure.

The medical history of patient R.W. (P7710) has also been published elsewhere (Handler, Isenberg & Greaves, 1981). He developed extensive maculo-papular urticaria pigmentosa over a 2-year period. Pruritus was intense and his uninvolved skin was dermographic. He had no systemic symptoms but his liver and spleen were enlarged. There were hepatic mast cell deposits, cirrhosis and portal hypertension. In addition, he developed polycythaemia rubra vera.

PUVA

8-Methoxypsoralen (methoxalen) was given orally in the conventional dose of 0·6 mg per kg body weight. Two hours later patients were exposed to UV-A (320–400 nm, peak at 365 nm) in PUVA 6000 and 4000 units (H. Waldmann, Germany). The initial dose of UV-A ranged from 1·5 to 2·5 J/cm^2. The dose was increased at subsequent visits with the aim of maintaining minimal erythema in the clinically uninvolved skin. PUVA treatments were given at least twice weekly at intervals of not less than 48 h.

The response to treatment was assessed by questioning concerning pruritus and spontaneous wealing, and by rubbing the skin in an attempt to provoke wealing in the lesions (Darier's sign) and in normal looking skin. Clinical impression was aided by examination of serial colour photographs.

Biopsies

Using 1% lignocaine without adrenaline injected around the biopsy site, as a local anaesthetic, skin biopsy specimens were obtained from representative lesions, usually on the upper thigh or lower trunk, before the start of treatment and after an average of twenty-seven PUVA exposures. Most samples were taken as a punch biopsy of 4 mm diameter. In six patients, samples of normal appearing skin, either adjacent to the lesion selected for biopsy, or from the contralateral side of the body, were also obtained before and after treatment. All these sites were accessible for UVR. In addition, in one patient, a lesion that had been shielded from UVR was also biopsied after PUVA. The choice of a representative lesion to biopsy after the course of treatment was facilitated because in any given area of the body the response of the lesions to PUVA was monomorphic.

The skin samples were divided and processed for microscopical studies and for determination of the histamine concentration. The sub-samples for histamine assay were stored at −20°C until required.

Mast cell counts

The material for microscopical analysis was processed and the mast cells were counted by techniques described previously (Eady *et al.*, 1979). Briefly, the tissues were fixed in half-strength Karnovsky fixative in cacodylate buffer, post-fixed in osmium tetroxide buffered with s-collidine, dehydrated in alcohol and embedded in Epon. One μm-thick vertical sections were stained with methylene blue and basic fuchsin. The mast cell population density was expressed as the number of cells per mm^2. For each biopsy sample, mast cell counts were made in sections from two blocks, and the average value calculated.

Electron microscopy

Either the tissue blocks that had been prepared for light microscopy (see above) were trimmed and used for electron microscopy, or separate portions of the biopsy specimens were, after fixation, dehydrated in alcohol and embedded in Araldite. Ultrathin sections were stained in uranyl acetate and lead citrate and examined in a JEOL 100CX or an AE1 EM6B electron microscope.

Histamine assay

The specimens for histamine assay were freeze-dried and weighed. The histamine was extracted with boiling distilled water and assayed using a modification (Eady *et al.*, 1979) of an automated fluorimetric technique (Evans, Lewis & Thomson, 1973). The mean histamine content of each biopsy specimen was obtained from triplicate estimations and the results are expressed as ng histamine/mg dry weight of the skin.

RESULTS

Dosage of UV-A

Patients received from fifteen to sixty-nine exposures (average twenty-seven) between the pre-treatment and the first follow-up biopsy (Table 1). The UV-A dose per exposure at the time of biopsy ranged from 2 to 12 J/cm^2 (average 6·4 J/cm^2). The variation in the dosage of UV-A was partly due to the different skin types of the patients. Minimal erythema was used as a guideline for dosimetry.

Clinical response to treatment

The therapeutic responses of the patients are summarized in Table 2. Most patients reported that after an initial exacerbation of pruritus related to the PUVA erythema, the amount of pruritus and spontaneous wealing was reduced. The tan produced by PUVA in the normal skin tended to mask the hyperpigmented macules, but as treatment progressed there seemed to be a genuine fading of the macules (Fig. 1) and the papules felt less infiltrated. Patients Y.W. and P.A. who were the most mildly affected by the disease were both exceptional in that their

TABLE 2. Clinical response

	Pruritus		Darier's Sign		
Patient	Before	After	Before	After	Pigmentation of lesion after treatment
T.K.	+ +	+	+	+/−	Partially faded
L.C.	+	−	+	−	Partially faded
Y.W.	−	−	−	−	Considerably faded
T.L.	+ +	−	+	+/−	Partially faded
D.P.	+ + +	−	+	+/−	Little change
R.W	+ + +	+ + +	+	+	No change
P.A.	+ +	−	+	−	Considerably faded
A.K.	+ + +	+ +	+	+/−	Partially faded

Pruritus: − = absent; + = slight; + + = moderate, + + + = severe.
Darier's sign: − = absent; +/− = equivocal; + = present.

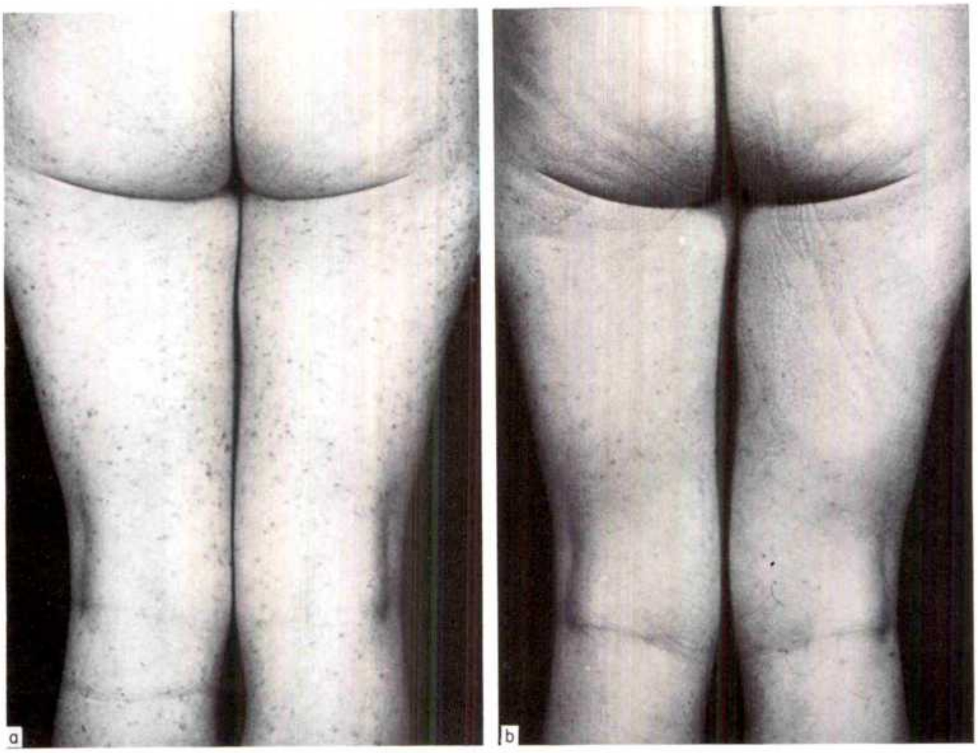

FIGURE 1. (a) Patient T.K. before PUVA therapy. Numerous pigmented maculo-papules are evident on the buttocks and thighs. (b) Same patient after twenty-two PUVA exposures. Although some pigmented lesions are still visible, most have faded. The skin generally has become tanned.

macules faded almost completely. Although the response of the lesions was monomorphic in a given anatomical site, there was regional variation and the areas which were less easily exposed to the UV-A rays, for example, the inside of the legs, tended to lag behind. In two patients, a 10 × 8 cm area of skin was shielded from UV-A with aluminium foil. After PUVA the lesions in the exposed skin were less noticeable than those in the unexposed sites but the number of lesions in both sites remained about the same. The response of the male patient with systemic mastocytosis was disappointing. Initially he reported reduced pruritus and wealing provided he also received treatment with hydroxyzine hydrochloride (Atarax®) which seemed to have no effect on its own. Eventually the symptoms worsened, some lesions became more infiltrated, and PUVA was abandoned when the full extent of his disease became apparent.

Mast cell counts

The result of the mast cell counts are given in Table 3. There was a wide variation in the mast cell count before the start of treatment, especially in lesional skin (range = 66–569 cells/mm^2; mean = 252·4 ± 190·3 s.d.). The mast cell count in uninvolved skin ranged from 32 to 92 cells/mm^2 (mean = 63·8 ± 22·3 s.d.). In patients Y.W. and D.P. the mast cell count in uninvolved skin was higher than the top end of the range (32 to 66 cells/mm^2) for arm skin in normal men (Eady *et al.*, 1979). The mean cell count in lesional skin was significantly higher than that of clinically uninvolved skin ($P < 0·05$; paired t test). The number of mast cells was not

TABLE 3. Mast cell counts (cells/mm^2) before and after PUVA

	Lesional skin		Uninvolved skin	
Patient	Before	After	Before	After
T.K.	118	89	51	47
L.C.	305	247	60	53
Y.W.	66	52	86	63
T.L.	102	75 (135*)	62	46
D.P.	569	880	92	44
R.W.	502	797	32	85
P.A.	209	240	n.d.	n.d.
A.K.	148	170	n.d.	n.d.
Mean ± s.d.	252·4 ± 190·3	318·8 ± 329·6	63·8 ± 22·3	56·3 ± 15·6

* Shielded skin.
n.d. = Not done.

significantly changed (using a paired t test) after PUVA in either the lesions or clinically uninvolved skin.

Electron microscopy

Comparing the pre-treatment lesional samples (seven patients) and non-lesional (four patients) with those obtained after PUVA, there was no difference in the shape or size of the dermal mast cells, or in the internal ultrastructure including the degree of granularity. In several lesions obvious degranulation with numerous extra-cellular granules was seen both before and after PUVA. In patients L.C., D.P. and R.W. (the last had systemic disease) the ultrastructure of the granules was abnormal before treatment and there was no change in the abnormality with treatment.

Histamine content

The results of the histamine measurements are given in Table 4. The lesional histamine concentrations in the six patients investigated ranged from 17·3 to 2279·7 nm/mg dry weight (mean = 514·6 ± 901·4 s.d.). Except for patient Y.W., the lesional histamine concentration was higher than the normal range (6·3–24·9 ng/mg), and the histamine concentration was higher in lesional skin than in uninvolved skin. PUVA treatment did not produce a consistent effect on the histamine concentration of lesional skin and there was no overall significant change ($P > 0{\cdot}2$; paired t test). The direction of the change of histamine concentration did not always correspond with that of the mast cell counts. The histamine concentration in the uninvolved skin did not change significantly after PUVA ($P > 0{\cdot}9$; paired t test).

Effect of shielding skin

An additional biopsy was taken from a foil-shielded lesion from patient T.L., after PUVA. The mast cell content in this lesion was higher than that of the UV-A exposed lesion (135 cells/mm^2 compared with 75 cells/mm^2 respectively, whereas the histamine level was lower (31·9 ng/mg compared with 46·8 ng/mg)).

TABLE 4. Histamine concentration (ng/mg dry weight) before and after PUVA

	Lesional skin		Uninvolved skin	
Patient	Before	After	Before	After
T.K.	33·4	32·3	16·2	13·2
L.C.	57·6	78·7	23·6	14·9
Y.W.	17·3	17·3	10·6	11·4
T.L.	29·6	46·8 (31·9)*	23·5	20·2
D.P.	2279·7	771·7	45·5	42·9
R.W.	669·7	201·4	45·4	62·2
Mean ± s.d.	514·6 ± 901·4	191·4 ± 291·9	27·5 ± 14·8	27·5 ± 20·6

* Shielded skin.

Follow-up

Patient T.K. received PUVA for a total of 6 months. Six months after stopping treatment she developed irritation around the wrists and ankles but the rash was still less prominent than it was before the start of PUVA and Darier's sign was negative. Patient L.C. discontinued PUVA after a total of 5 months. Ten months later Darier's sign became positive again but otherwise her clinical condition did not deteriorate. To date, patient Y.W. has had three courses of PUVA, and her macules have faded after each one. Her first remission lasted 5 months without treatment and her second lasted 6 months. Patient T.L. discontinued PUVA after a total of 6 months when there had been considerable improvement. Seven months later her condition deteriorated and she was referred for a further course of treatment. Miss D.P. stopped PUVA after sixteen treatments. One year later the lesions were again raised and infiltrated. In the case of R.W. PUVA was stopped after a total of 9 months due to lack of efficacy. Patient P.A. continued treatment for 16 months, but the disease relapsed over the following 4 months and photochemotherapy was recommenced. One year after starting PUVA, patient A.K. was still on treatment and symptomatically she was better although she still complained of itching and macules were still visible. The mast cell count in lesional skin at this time was 124 cells/mm^2 as compared with 148 cells/mm^2 before the start of treatment.

DISCUSSION

Our clinical findings support previous reports about the ability of PUVA to suppress some of the cutaneous manifestations of urticaria pigmentosa. However, improvement is slow and only partial, and the disease tends to relapse once the treatment is discontinued. PUVA treatment therefore seems indicated only in patients who are severely inconvenienced by the disease, particularly if it has failed to respond to conventional therapy. The response of patients with very severe cutaneous involvement may be disappointing. In addition, the carcinogenic potential of PUVA should be borne in mind in weighing the advantages against the risks of treatment.

Proliferation of dermal mast cells plays a central role in the pathogenesis of urticaria pigmentosa. Mast cells synthesize and release many products including vasoactive agents, chemotactic mediators, proteoglycans and enzymes (Wasserman, 1980) but it is widely believed that pruritus, flushing and wealing are due to histamine release. Granerus *et al.* (1981) investigated the effect of PUVA in three patients with urticaria pigmentosa and systemic mastocytosis. A conspicuous reduction in the number of dermal mast cells was reported although no quantitative data were published. In all three patients a statistically significant decrease in the urinary excretion of 1-methyl-4-imidazoleacetic acid, a major histamine metabolite, was noted. These changes coincided with a reduction in pruritus and partial fading of the lesions. The authors suggested that the beneficial effect of PUVA in their patients was due to diminished histamine turnover in the skin secondary to a reduced number of mast cells. Using different techniques we did not detect a significant effect of PUVA on mast cell numbers, in both lesional and uninvolved skin. Our PUVA protocols and clinical findings were broadly similar so technical differences may account for different results.

PUVA might interfere with the release but not the synthesis of histamine by 'stabilizing' the membranes involved in degranulation. This change would not have been detected by our methods, although the reduction in the wealing response to friction in both lesional and clinically uninvolved skin might suggest a decrease in mast cell degranulation.

In 1961, Valtonen studied the effect of UVR on mast cell counts in mouse ear. He showed that there was a significant increase in the mast cell population of skin irradiated continuously for 32 days with short-wave UVR or with combined mid- and long-wave UVR. With long-wave UVR on its own the mast cell counts remained unchanged. We know of no information about the effect of psoralens plus UV-A on the mast cell count in the skin of experimental animals or normal human subjects. We found there was no apparent effect of PUVA in the 'normal' skin of patients with urticaria pigmentosa.

The possibility that PUVA influences vascular responsiveness to histamine must also be considered. Ashton & Greaves (1983) found that neither UV-B irradiation nor PUVA treatment has any detectable effect on the erythema or weal response to intradermal injections of histamine in doses of 1·8–1800 nmol. Fjellner & Hägermark (1982a) noted that PUVA and UV-B exposure significantly alleviated the itch and flare evoked by intradermal injections of the mast cell degranulator compound 48/80, and postulated that UVR might either inhibit mast cell release mechanisms or deplete mast cell histamine stores. They also found (Fjellner & Hägermark, 1982b) that pre-exposure of rat peritoneal mast cells to UV-B (but not UV-A) inhibited histamine release induced by compound 48/80. There is no information on the influence of PUVA in this *in vitro* system.

The relief of pruritus which occurs without change in histaminuria in patients treated with disodium cromoglycate for systemic mastocytosis (Soter *et al.*, 1979) may be due to interaction of the drug with peripheral nerve fibres (Altounyan, 1980). PUVA treatment is known to alter the ultrastructure of cutaneous nerves (Kumakiri & Hashimoto, 1978) and cause deposition of amorphous material around cutaneous blood vessels of psoriatic lesions (Hashimoto *et al.*, 1978). If these changes occur in the skin of patients treated with PUVA for urticaria pigmentosa then they could conceivably be implicated in the response to the treatment.

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A randomized trial comparing cadexomer iodine and standard treatment in the out-patient management of chronic venous ulcers

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SUMMARY

Ninety-three patients with treatment-resistant venous ulcers were included in a multicentre randomized trial to compare cadexomer iodine and the standard treatment used in each centre combined with compression bandages, in healing venous ulcers. The mean duration of ulcers before the trial was more than 2 years. With standard treatment the mean ulcer size increased slightly during the 6-week trial whereas with cadexomer iodine the ulcer size was significantly reduced. Cadexomer iodine was more effective than standard treatment for reduction of pain, removal of pus and debris, removal of exudate, stimulation of granulation and reduction of surrounding erythema. Bacterial infection of ulcers increased or did not change during treatment with the standard therapy whereas cadexomer iodine significantly reduced infection with *Staphylococcus aureus*, *Pseudomonas aeruginosa* and other pathogenic organisms. A correlation was seen between the time taken to reduce or eliminate infection with *Staphylococcus aureus* and rate of ulcer healing. Four patients complained of transient pain in the ulcer after application of the cadexomer iodine. It is concluded that cadexomer iodine increased the rate of healing of infected chronic venous ulcers.

Leg ulcers associated with venous hypertension are common (Coon, Willis & Keller, 1973; Gjöres, 1956). Almost any bland dressing together with compression bandages may be

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efficacious for the treatment of venous ulcers, but there exists a population of patients ulcers do not heal with currently available therapy. We have studied a new treat cadexomer iodine (Iodosorb®, Perstorp AB), in a ten-centre randomized trial in chronic v ulcers. Cadexomer iodine is a dry powder consisting of biodegradable spherical microb These are insoluble but hydrophilic and contain 0·9% iodine physically immobilized v their matrix. Exudate is absorbed and particulate matter is removed from the ulcer surfa the action of the microbeads and simultaneously iodine is released. It is claimed that this sy maintains a combined debriding and bactericidal effect at the ulcer surface.

METHODS

Ninety-three male and female out-patients with chronic infected venous ulcers were select the trial. Ulcers were required to have a minimum diameter of 2 cm and minimum area of and to have failed to respond to current treatments. Patients with a history of iodine sensi and patients with peripheral arterial disease evidenced by clinical appearance of the peripheral pulses and in doubtful cases, reduced toe or ankle pressures, were excluded.

Ulcers were classified as deep, superficial or very superficial based on clinical assessment ulcer size was measured by planimetry after tracing the outline of the ulcer or measurin greatest diameters. All ulcers were photographed against a ruler scale before treat commenced. Swabs were taken from the edges of the ulcers for bacterial culture. Bacteria quantified as absent, +, ++ or +++ according to standard plating techniques for organism type. Patients were allocated blindly and at random to the standard treatment cadexomer iodine. Assessments were performed after 1, 2, 4 and 6 weeks of treatment. At visit the ulcer was measured and photographed, swabs were taken for bacterial culture ar assessment was made on an analogue scale of the quantity of pus and debris on the ulcer sur quantity of exudate and of granulation tissue in the ulcer base and the degree of oedema erythema of the surrounding skin. At each centre the same observer always made observations. Ulcer pain was recorded on a similar scale.

Treatment

All patients were treated with compression bandages applied by a visiting nurse at home o hospital clinic. Some patients were trained to apply their own bandages.

Cadexomer iodine. After the ulcer had been gently washed in running water cadexomer io powder was applied to a depth of approximately 3 mm and covered with a dry dressing.

Standard treatment. Before entry to the trial a large variety of local treatments was in including enzyme preparations, dextranomer, fucidic acid, trypure powder, polymyxin silver nitrate. The ulcers had failed to respond to these and for the duration of the trial a diffe regime of management was used. Each day the ulcers were gently cleaned with dilute hydro peroxide or dilute potassium permanganate baths and non-adherent dressings were app Paraffin-impregnated dressings were most commonly used, but saline dressings and b ointments were also used occasionally. In the event of specific difficulties or failed response physician was permitted to modify the regime of standard treatment depending upon appearance of the ulcer and condition of the surrounding skin. Additional agents emplo included merbromine, Salvstrumpa® (this consists of a specially designed support stocking inner layer of which contains ointment with a base of lanolin, vaseline and paraffin contai zinc and sodium borate) and systemic antibiotics. In both treatment groups, during the first few days of the trial when exudate was heavy, the dressing could be changed twice daily, but this was seldom necessary and for the remainder of the trial dressings were changed daily.

RESULTS

Ninety-five sets of patient data were received for evaluation, of which twenty-one were excluded from the statistical analysis (Table 1). Evaluation of the results of excluded patients from the standard group showed a mean increase of ulcer size of 10·9 cm^2 and in the cadexomer iodine group a mean decrease of 4·3 cm^2.

No significant pre-treatment differences were observed between the groups (Table 2).

The mean ulcer size was significantly reduced after 1 week of treatment with cadexomer iodine and continued to decrease during the 6 weeks of the trial. With standard teatment a small decrease in ulcer size was observed during the first week of treatment which was not maintained. The percentage decrease of ulcer area was significantly greater with cadexomer iodine at each observation time (Fig. 1). After 6 weeks of treatment the mean ulcer size in the cadexomer iodine group had reduced by 34% and in the standard group it had increased by 5%.

Both treatments were associated with a significant reduction of pain during the trial but the effect of cadexomer iodine occurred earlier and was significantly greater ($P < 0·01$) than that of the standard treatment.

A significant debriding effect on the ulcers was apparent in each group within 1 week of starting treatment. The cleansing effect of cadexomer iodine was significantly greater than that of the standard treatment ($P < 0·005$) (Table 3).

The effect of cadexomer iodine on stimulating granulation and reducing erythema of surrounding skin was significantly greater than the standard treatment. Both treatments significantly reduced oedema of the surrounding skin although the effect of standard treatment was observed later.

Bacteriology

The most frequently isolated organism was *Staphylococcus aureus* which was present before treatment in 77% of the patients. In two patients of the standard group this organism was reduced or eliminated during treatment compared with seventeen patients from the cadexomer iodine group ($P < 0·001$, Chi-square test with Yates' correction).

TABLE 1. Drop-outs and patients excluded from the statistical analysis

Reason for dropout or exclusion	Standard treatment	Cadexomer iodine
Did not meet selection criteria	3	4
Rash	—	1
Holidays	—	2
Beta-haemolytic *Streptococcus* infection	4	2
Squamous cell carcinoma diagnosed	1	—
Dramatic increase in ulcer size	1	—
Missing information	—	2
Recurrence of ulcer pain	—	1
Total	9	12

TABLE 2. Pre-treatment group comparability

	Standard treatment	Cadexomer iodine
Sex	M = 8	M = 10
	F = 28	F = 28
Age*	72·1 ± 3·54	68 1 ± 1·99
Weight*	75 5 ± 5 92	78·8 ± 5 49
Duration of ulcer* (months)	22 2 ± 14·27	26·5 ± 18 26
Type of ulcer		
Venous	30	37
Mixed	6	1
Size of ulcer (cm^2)	34 ± 5·7	20·1 ± 4 4
Depth of ulcer		
Deep	12	11
Superficial	23	26
Very superficial	1	1

* Mean ± s.e.m.

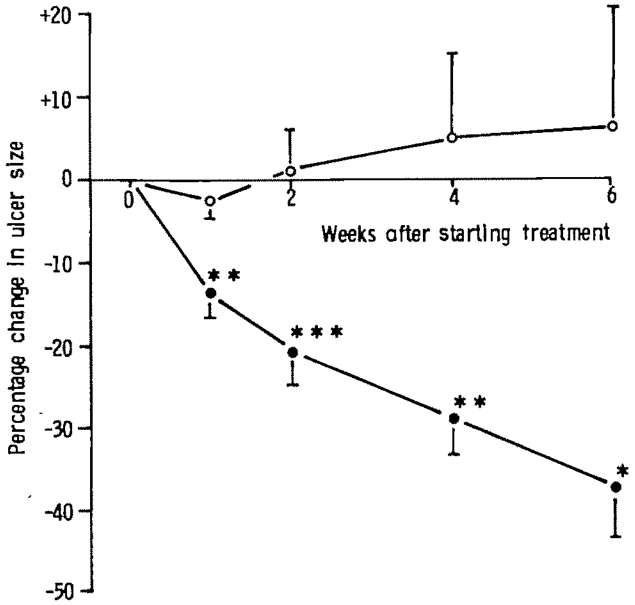

FIGURE 1. Mean ± s.e.m. percentage change in ulcer area in thirty-six patients on standard treatment (○) and thirty-eight patients on cadexomer iodine (●). Significance of differences between the groups: *$P < 0·02$, **$P < 0 01$, ***$P < 0 005$ (Student's t test). The reduction from baseline is significant in the cadexomer iodine group at 1, 2, 4 and 6 weeks ($P < 0 002$). Wilcoxon matched pairs signed-ranks test, 2-sided.

TABLE 3. Responses of pain, pus and debris, exudate, granulation, erythema and oedema to cadexomer iodine (CI) and standard treatment (Mean ± s.e.m. of visual analogue scales)

		Pre-treatment	1 Week	6 Weeks
Pain	Standard	33 ± 4·3	29 ± 4·1	23 ± 3·7* xx
	CI	32 ± 4·7	27 ± 4·6***	10 ± 2·5***
Pus and Debris	Standard	33 ± 4·4	30 ± 3·9*	22 ± 3·4*** xxx
	CI	31 ± 3·1	23 ± 2·8***	8 ± 1·6***
Exudate	Standard	35 ± 4	32 ± 3·5*	23 ± 3·6*** xxx
	CI	37 ± 3·5	27 ± 3·1***	11 ± 1·5***
Granulation	Standard	47 ± 4·2	51 ± 3·9**	60 ± 4·6*** x
	CI	39 ± 5·4	47 ± 5·0***	76 ± 4·0***
Erythema	Standard	27 ± 2·7	26 ± 2·7	23 ± 2·9 xxx
	CI	28 ± 2·9	27 ± 2·7	13 ± 2·0***
Oedema	Standard	26 ± 3·9	22 ± 3·2	20 ± 3·0*
	CI	23 ± 3·2	21 ± 3·0	8 ± 2·0***

Significance of difference between CI and standard treatment after 6 weeks of treatment: x = $P < 0{\cdot}05$; xx = $P < 0{\cdot}01$; xxx = $P < 0{\cdot}005$ (Student's t test). Significance of change from baseline: *$P < 0{\cdot}05$; **$P < 0{\cdot}01$; ***$P < 0{\cdot}005$ (Test of paired comparisons). Cadexomer iodine group = 38 patients. Standard treatment group = 36 patients

A colony count of + + + using a standard plating technique was defined as infection. In the standard group in no case was staphylococcal infection cleared and in eighteen cases infection persisted or new infection occurred during treatment. With cadexomer iodine in sixteen patients infection was cleared and in seven patients existing infection was not cleared or a new infection occurred ($P < 0{\cdot}001$, chi-square test with Yates' correction). There were six patients in the standard group in whom infection with *Pseudomonas aeruginosa* occurred during treatment or in whom existing infection persisted, whilst in one patient infection improved. With cadexomer iodine no new infection with *Pseudomonas aeruginosa* occurred and in all three infected cases pre-treatment infection improved or cleared during treatment ($P < 0.05$, Fisher's exact test).

The other most commonly occurring pathogenic organisms were beta-haemolytic *Streptococcus*, *Proteus*, Enterobacteria and *Klebsiella*. Cadexomer iodine was significantly more effective than the standard treatment against these organisms ($P < 0{\cdot}001$, chi-square test).

Side effects

One patient on standard treatment and four on cadexomer iodine complained of pain after application of the dressing which commenced between 10 min and 2 h after changing the dressing and which subsided after 30–60 min. One patient had itching around the ulcer which subsided after 2 weeks without interruption of treatment. One other patient dropped out of the study because of a skin rash. Skin tests showed she was not sensitive to cadexomer iodine but to coal tar derivatives probably present in her soap.

Routine laboratory tests including haematology, urea and electrolytes and liver function tests were performed before treatment and after 6 weeks. No significant changes were seen other than in the white cell count, which was reduced in both groups in relation to the healing of the ulcers. Thyroxine, tri-iodothyronine, serum thyroxine binding protein and thyroid index did not change significantly during the study in either group.

DISCUSSION

The published literature is devoid of properly controlled studies on the healing of chronic venous ulcers and the absence of any reliable methods for assessment of ulcer healing has been stated as a reason for not performing controlled trials (Allen & Turner, 1979). However, it was found in this trial that direct measurement of ulcer size by tracing and planimetry and with a rule, confirmed for the record by photography against a centimetre scale, permitted the demonstration of a significant reduction in ulcer size within 1 week of starting treatment and a significant difference between the treatment groups. In general, venous ulcers which have been present for only a short time heal within a few weeks with currently used treatments, while ulcers which have been present for several months are usually much more resistant to healing. In this trial the duration of ulcers before treatment did not correlate with ulcer size, and the size of ulcer before treatment did not correlate with healing; the best ten responders in each group in terms of absolute and percentage change of ulcer size had a mean ulcer size before treatment higher than the mean size for the total population, and some of the smaller ulcers were among the most resistant to treatment. A number of factors have been examined for relationships with response to treatment; ulcer duration has proved the most significant (unpublished information).

The significance of bacteria in chronic ulcers has not been clearly defined and it is generally considered that many of the organisms found in ulcers have no adverse effect on healing. A significant correlation was observed between clearing of infection with *Staphylococcus aureus* and rate of ulcer healing suggesting that its presence delays healing. Animal studies have demonstrated a relationship between infection with *Pseudomonas aeruginosa* and rate of healing of standard burns (J.C.Lawrence, personal communication).

The effect of cadexomer iodine on pain and its ease of application and removal, even by elderly patients, gave high acceptance by patients and nurses. However, transient pain in the ulcer after application of cadexomer was reported by four patients.

Before embarking on this trial the protocol was discussed at length, in particular concerning choice of the standard treatment. Experience with topical iodine had shown that its bactericidal action in solution is only transient and the other logical choice for control treatment, dextranomer, had already been used without success on many of these patients and did not meet the criterion of best local treatment for any of the participants. The regime eventually chosen was remarkably similar to that recommended in a recent editorial (*British Medical Journal*, 1982).

The major objectives in the local treatment of venous ulcers are to prevent accumulation of exudate and crusts and to create an infection-free environment so that epithelial cells can cover the ulcer. Any dressing should not become incorporated into the wound unless it is biodegradable and it should not tear off fragile new epithelium when removed. Moreover, the dressing should provide a stable, non-adherent contact layer which allows humidity as well as gas exchange, and a better absorptive mass superficially (*British Medical Journal*, 1979). Any system for preventing or treating local infection should not be susceptible to development of resistant organisms and it should not involve chemicals which are toxic or which kill or inhibit the formulation of new cells.

Cadexomer iodine powder consists of spherical microbeads. One gram can absorb up to 7 ml of fluid. Pore size increases with uptake of fluid and iodine is slowly released at the wound surface. Iodine has been used for many years for the local treatment of wounds and ulcers but its bactericidal effect is transient because it is rapidly inactivated by protein. A local slow release system provides a prolonged antibacterial action. The antibacterial action, biodegradability and greater absorptive capacity distinguish cadexomer iodine from dextranomer (Debrisan), with which viable organisms may persist in the dressing at a higher concentration than in the ulcer (Dagher, Alangi & Smith, 1978).

Many physicians concerned with the treatment of venous ulcers feel that with appropriate dressings, venous support and instructions on everyday activities most ulcers will heal. However, this study revealed a substantial population of patients with ulcers which had been present for a mean duration of more than 2 years and which had a mean area of more than 20 cm^2. With the standard treatment almost half of the ulcers increased in size despite compression bandages and local treatment, whereas 84% responded to cadexomer iodine. These results suggest that in out-patient treatment of chronic venous ulcers, despite adequate compression bandages, the choice of topical agent may significantly influence healing.

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Case Reports

The vascular lesions associated with skin necrosis in renal disease

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SUMMARY

Three renal allograft recipients and one uraemic patient presented with skin necrosis. In all cases, the subcutaneous arteries and arterioles were narrowed or occluded by mural calcification with or without intimal fibrosis.

A review of the literature shows that uraemic patients or allograft recipients with skin necrosis involving the trunk or thighs have a poor prognosis while recovery is common in patients with more peripheral lesions. The aetiology of the arterial lesions is unknown. Their relationship to renal disorders and/or hyperparathyroidism is uncertain and therapy remains unsatisfactory.

Arterial calcification is common in patients with chronic renal failure (Tatler *et al.*, 1973; Meema, Oreopoulos & DeVeber, 1976). It seldom resolves, even after a successful renal transplantation (Alfrey *et al.*, 1968; Meema *et al.*, 1976).

The calcific deposits may be localized in the intima or in the media. Intimal calcification is found in patches at the base of atheromatous plaques particularly in the aorta, its major branches, and in the cerebral arteries. Medial calcification is characterized by ring-like deposits in small to medium-sized arteries ('Monckeberg's sclerosis'). This type of calcification which is common in uraemia (Parfitt, 1969) is generally regarded as dystrophic rather than metastatic in origin, i.e. it is believed to be secondary to degenerative rather than biochemical changes. In spite of spectacular radiological appearances, the medial lesions (Parfitt, 1969) appear clinically harmless and do not lead to ischaemic changes.

A relatively rare syndrome of skin necrosis and/or peripheral gangrene has been reported in uraemic patients and in renal allograft recipients (Bryant & White, 1898; Brown & Ginsburg, 1940; Richards, 1951; Anderson, Stewart & Piercy, 1968; Rees & Coles, 1969; Friedman, Novack & Thomson 1969; Richardson *et al.*, 1969; Goodhue, Davis & Porro, 1972; Rosen *et al.*,

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1972; Cooksley & Craswell, 1972; Geis *et al.*, 1973; Conn *et al.*, 1973; Koranda *et al.*, 1974; Hallgren *et al.*, 1975; Huertas, Maletz & Weller, 1976; Gipstein *et al.*, 1976; Blumberg & Weidmann, 1977; Peterson, 1978; Kossard & Winkelman, 1979; Perloff *et al.*, 1979; Collier & Mitch, 1980). In this paper, a further four patients with this syndrome are reported, together with a detailed description of a third type of vascular calcification. An attempt has been made to define the role of the vascular lesions in the development of skin necrosis.

CASE REPORTS

Case 1

H.B., a 49-year-old woman with renal failure attributed to chronic pyelonephritis, commenced haemodialysis in January 1969 and received a cadaveric renal transplant in October 1969.

In December 1969 she presented with pain and violaceous discoloration of both thighs. During the week after presentation, the affected skin area became progressively darker, the lesions spread proximally and fresh areas of involvement occurred over the buttocks and lower abdomen. The patient required frequent narcotic medication for pain relief. Serum creatinine was 0·07 mmol/l, serum calcium 2·55 mmol/l and serum inorganic phosphate 0·6 mmol/l. Roentgenograms showed subperiosteal erosions of the phalanges and extensive vascular calcification involving vessels of all sizes. Debridement of the necrotic areas was performed and on 11 February 1970, three and a half hyperplastic parathyroid glands were removed. The patient developed left hemiparesis on 5 March 1970 and died of a cardiac arrest on 8 April 1970. The autopsy findings are described below.

Case 2

C.C., a 39-year-old housewife whose renal failure was attributed to chronic glomerulonephritis, commenced chronic peritoneal dialysis in January 1972 and received a cadaveric renal transplant 3 months later. The graft was removed on the ninth post-operative day because of acute rejection.

Maintenance haemodialysis was commenced in July 1972 and a second cadaveric kidney was transplanted in May 1978. The serum creatinine stabilized at about 0·2 mmol/l for the first 7 months but then gradually rose. On 14 June 1980 she presented with dusky painful areas on both buttocks. Four days later the skin over the affected areas became ulcerated and further discolored lesions extended proximally to the trunk and distally to both thighs (Fig. 1). The lesions were very painful and the patient required frequent narcotic medications. On presentation, serum creatinine was 0·99 mmol/l, serum calcium 2·27 mmol/l, serum inorganic phosphate 1·91 mmol/l, serum alkaline phosphatase 83 U/l, and serum immunoreactive parathyroid hormone (iPTH) 2·1 ng/ml (normal less than 0·5 ng/ml).

Roentgenograms showed extensive calcification involving vessels of all sizes which had progressed since a previous examination three months earlier. The roentgenograms also showed tumoral calcification over the greater trochanter of both femurs.

A left iliac crest biopsy showed an increase in resorption surfaces and osteoclasts. Maintenance haemodialysis was recommenced and on 4 July 1980 three hyperplastic parathyroid glands weighing 350 mg were removed. Post-operatively the skin lesions continued to progress and the patient died 2 weeks after parathyroidectomy. The pathological findings are described below.

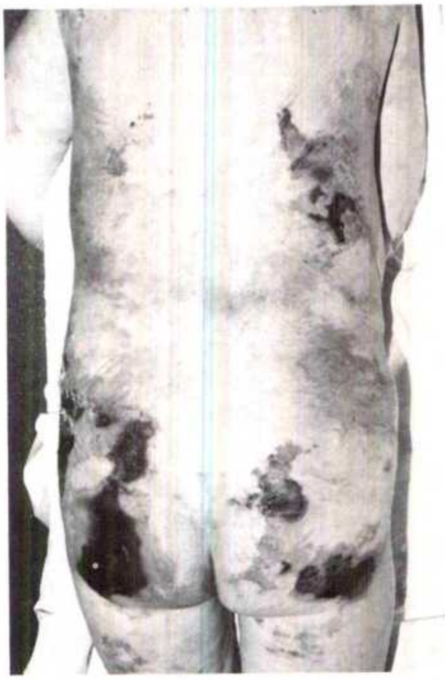

FIGURE 1. Appearance of the skin in patient 2 at autopsy. The irregular, sharply demarcated margins of the gangrenous areas are characteristic of this syndrome.

Case 3

N.B., a 43-year-old housewife with renal failure attributed to chronic glomerulonephritis, commenced haemodialysis in October 1970 and received a cadaveric renal transplant in December 1970. Serum creatinine was normal until 1974 when it started to rise gradually reaching 0·84 mmol/l in November 1978. At that time she complained of pain in her back and legs. Serum calcium was 2·26 mmol/l, inorganic phosphate 0·96 mmol/l, alkaline phosphatase 805 U/l and iPTH 4·75 ng/ml. She was given 50,000 units ergocalciferol per day for 6 months.

Maintenance haemodialysis was recommenced in June 1979 and 2 weeks later she developed painful ulcers in both legs. There was no history of claudication and examination showed normal peripheral pulses. Serum calcium was 2·21 mmol/l, serum inorganic phosphate 1·58 mmol/l, serum alkaline phosphatase 352 U/l, serum iPTH 2·0 ng/ml. Roentgenograms showed subperiosteal erosions of the fingers and extensive calcification involving vessels of all sizes which had progressed since a previous examination 3 months earlier. A left iliac crest biopsy showed increases in resorption surfaces, osteoclast counts and endosteal fibrosis. New skin ulcers appeared during the next few weeks, all of them confined to areas below the knees.

On 12 September 1979 an appendicectomy was performed and over the subsequent 6 weeks (three of which were spent in hospital) the ulcers gradually healed. Four hyperplastic parathyroid glands weighing 850 mg were removed in November 1979. She has since remained well on haemodialysis and vitamin D replacement with no recurrence of her leg ulcers.

Case 4

G.H., a 53-year-old woman with advanced renal failure attributed to analgesic nephropathy, presented in October 1979 with multiple painful skin ulcers in the region of both ankles. She did not have claudication and all her peripheral pulses were normal. Serum creatinine was 1·09 mmol/l, serum calcium 2·38 mmol/l, serum inorganic phosphate 3·48 mmol/l, serum alkaline phosphatase 585 U/l and serum iPTH 5·3 ng/ml. Roentgenographs showed subperiosteal erosions of the phalanges and widespread calcification involving vessels of all sizes. Five weeks after the appearance of the leg ulcers, she had a cardiac arrest during the administration of intravenous ampicillin and died 4 days later. The pathological findings are described below.

Pathological findings

Skin biopsies were obtained in all four cases. The specimens were fixed in 10% buffered formalin and sections were stained separately with haematoxylin and eosin, alizarin red-S (to demonstrate calcium), and the Verhoeff-Van Gieson stain (for elastic tissue and muscle). Autopsy was performed in the three patients who died, and sections from various organs were examined specifically for ectopic calcification, arterial and arteriolar lesions. Specimens obtained at parathyroidectomy in cases 1–3 were similarly reviewed.

In each case the skin showed necrosis of the dermis and subcutaneous fat with narrowing and occlusion of the underlying subcutaneous arteries and arterioles caused either by intimal fibrosis (Fig. 2) associated with mural calcification (Cases 1–3), or by massive calcification alone (Case 4).

Small subcutaneous arteries and arterioles which were not occluded showed a range of changes. Those without mural calcification were normal. Some showed irregular and asymmetrical calcification in the region of the internal elastic lamina as the only abnormality (Fig. 3). In other instances internal elastic laminar calcification was accompanied by medial calcification and intimal fibrosis. The degree of intimal fibrosis and its distribution in the circumference of the vessel were parallel to the degree of mural calcification (Figs 4 and 5).

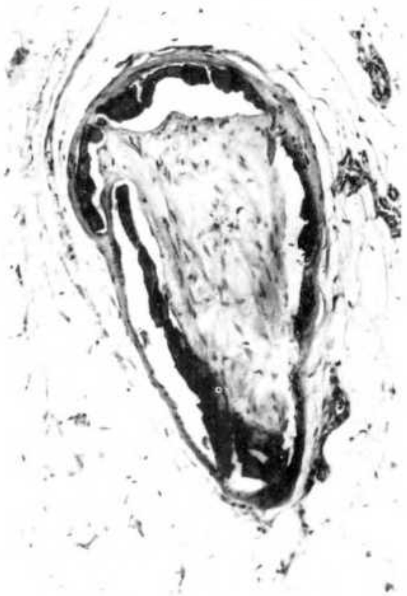

FIGURE 2. Small subcutaneous artery with heavy mural calcification (case 1). The lumen is obliterated by fibrous tissue containing myointimal cells. (H & E., × 90).

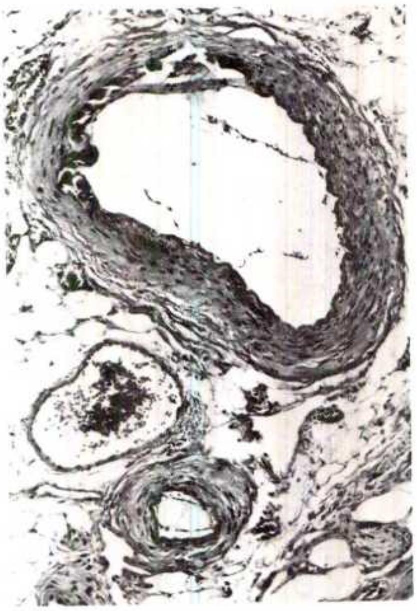

FIGURE 3. Subcutaneous arteriole (below) shows calcification of internal elastic lamina with normal intima (case 1). Small artery (above) shows calcification of internal elastic lamina and adjacent media, with early intimal change. (H & E, × 70).

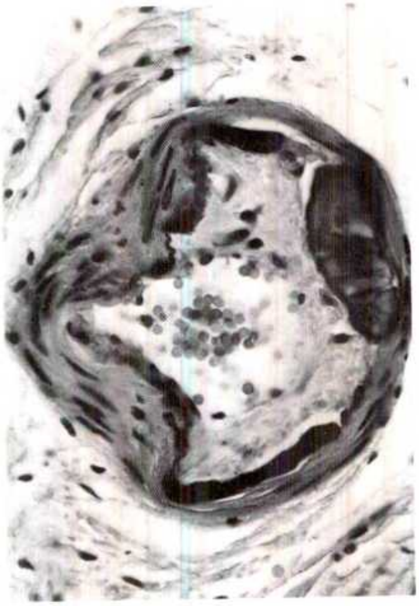

FIGURE 4. Subcutaneous arteriole with asymmetrical calcification of the internal elastic lamina (case 1). Intimal fibrosis is most marked adjacent to the most heavily calcified areas. (H & E, × 290).

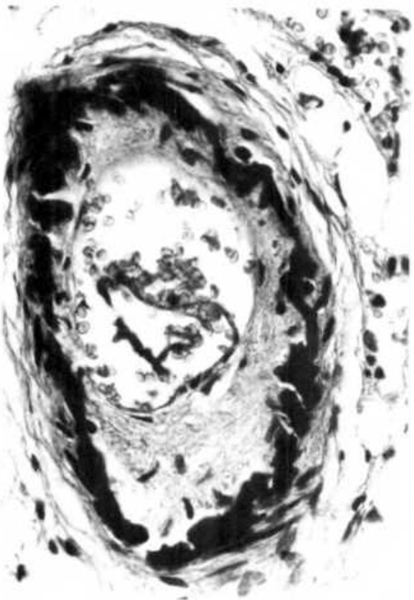

FIGURE 5. Subcutaneous arteriole with heavy circumferential calcification related to the internal elastic lamina, and concentric intimal fibrosis (case 1). (H & E, × 290).

Thrombi were absent though occasional vessels in case 2 contained small double lumina. No foam cells were seen in the intima. There was no evidence of vasculitis and the veins were normal.

The extent of the skin lesions correlated well with the severity and distribution of abnormalities in the subcutaneous arteries and arterioles, though similar non-occlusive lesions were found in subcutaneous arterioles of intact skin adjacent to the ulcerated areas.

Narrowing and occlusion of small arteries and arterioles by mural calcification and intimal fibrosis were also demonstrated in the peri-parathyroid adipose tissue in cases 1 and 2 and, in case 1, in myometrium and adjacent to a pulmonary infarct. Small arteries (up to 1·4 mm diameter) and arterioles of skeletal muscles were affected in cases 1 and 2, associated in case 1 with necrotic, atrophic and regenerating muscle fibres without inflammation or fibrosis. Calcification of the internal elastic lamina of arteries, without occlusion, was present in sections from various organs in all three autopsied cases.

Minor degrees of extravascular calcification were seen in a few pulmonary alveolar septa and the splenic capsule (case 1), in peri-parathyroid adipose tissue, a few myocardial fibres and bronchial cartilage (case 2), and in the posterior cusp of the mitral valve (case 4). The kidneys of the three autopsied patients showed calcification associated with scarring.

Atheromatous lesions were present but were not particularly severe. Some coronary atheroma was present in each of the three patients who came to autopsy while the aorta and the left renal artery were involved in case 4. The other larger arteries appeared macroscopically normal.

Sections were examined of apparently normal skin adjacent to various neoplasms from four other renal allograft recipients who had no skin necrosis but whose radiographs showed extensive calcification of large and medium-sized arteries. Neither vascular calcification nor fibrosis was found in the subcutaneous arteries or arterioles of these patients.

DISCUSSION

The occlusive vascular lesions demonstrated in the small arteries and arterioles in these biopsies are unusual and are clearly different from both intimal atheroma and medial calcification. Arteriolar calcification with intimal proliferation has previously been reported in association with skin necrosis in uraemia (Richardson *et al.*, 1969; Rosen *et al.*, 1972; Cooksley & Craswell, 1972; Conn *et al.*, 1973; Blumberg & Weidmann, 1977) though it is impossible to prove from studies of this nature that these lesions are related either to renal disease or to the development of skin necrosis.

The observation that calcification may occur without luminal fibrosis, while fibrosis occurs only in the presence of vascular calcification suggests the following sequence. Calcium deposition initially occurs in and around the internal elastic lamina, eventually destroying this structure. Calcification then encroaches on and eventually replaces the media. The intima remains normal until the internal elastic lamina is heavily calcified. Then a loose-textured fibrous intimal proliferation develops which ultimately results in occlusion of the lumen.

The correlation between the severity and distribution of these vascular lesions and the extent of skin necrosis, as well as the fact that arteriolar calcification could be present in the subcutis of intact skin, suggest that arterial and arteriolar damage preceded and possibly induced the skin lesions.

The pathogenesis of the syndrome of arteriolar calcification and peripheral necrosis has been compared to the process of 'calciphylaxis' described by Selye in experimental animals (Selye, 1962). Calciphylaxis consists of the administration of a 'systemic calcifying factor', followed by a 'critical period' during which several non-specific 'challengers' cause ectopic calcification.

The calciphylactic lesions, as described by Selye (1962) differ in several respects from those found in uraemic patients with skin necrosis. Experimental calcinosis is almost invariably extravascular while arteriolar or arterial calcification is not present. Serum calcium and inorganic phosphate were not measured in Selye's rats. However, the 'systemic calcifiers' employed (dihydrotachysterol, vitamin D_2, vitamin D_3, calcium salts, bovine parathyroid hormone, sodium acetylsulphathiazole, bilateral or partial nephrectomy, phosphate salts) are likely to have resulted in gross hypercalcaemia or hyperphosphataemia. It is therefore likely that what Selye demonstrated was largely the result of metastatic calcification due to an increased calcium × phosphate product.

Hyperparathyroidism was present in all our patients and in most of the cases reported in the literature. Skin necrosis has also been described in a few patients with primary hyperparathyroidism (Rogers *et al.*, 1947; Ellis & Barr, 1951; Bogdonoff *et al.*, 1956; Winkelmann & Keating, 1970). However, in some patients with anteriolar calcification and skin necrosis there was no evidence of hyperparathyroidism (Kossard & Winkelmann, 1979; Golitz, Field & Island, 1972).

An increased calcium × phosphate product might be important in the pathogenesis of arteriolar calcification. Control of serum phosphate, as illustrated by case 3 in our series and in two other patients reported in the literature (Huertas *et al.*, Gipstein *et al.*, 1976) may have been associated with healing of the skin lesions. However, an increased calcium × phosphate product was not demonstrable at the time of presentation in three of the four patients described in this paper or in most of the reported renal transplant patients with this condition.

Parathyroidectomy has been advocated for this potentially fatal condition (Gipstein *et al.*, 1976) but a review of the literature suggests that the outcome of this operation is unpredictable.

The forty-three previously reported patients and the four patients in this report may be divided into two groups according to the areas of skin involved. In twenty-two patients with skin

TABLE 1. Clinical data of patients with proximal skin necrosis

Author	Year	Case No.	Sex	Age	Status*	Areas of skin involvement	No. of parathyroid glands removed	Outcome
Anderson *et al.*	1968	1	F	62	PD	Thighs, knees	—	Died
Richardson *et al.*	1969	1	F	29	PD	Buttocks, thighs, legs, feet, fingers	—	Died
		2	F	36	4 months post Tx	Buttocks, thighs, legs, left arm	2	Died
Goodhue *et al.*	1972	1	M	25	11 months post Tx	Trunk, thighs	—	Died
Cooksley & Craswell	1972	1	F	34	PD	Buttocks, thighs	—	Died
		2	F	42	PD	Thigh	—	Died
Geis *et al.*	1973	1	?	?	Post Tx	Widespread	Subtotal	Died
Conn *et al.*	1973	1	F	23	6 months post Tx	Right hand, fingers, right buttock and thigh	3½	Died
Koranda *et al.*	1974	1	?	?	Post Tx	Abdomen, buttocks, legs	4	Died
Huertas *et al.*	1976	1	M	47	Uraemic	Trunk, thighs, shins	—	Died
		2	M	45	HD	Thighs, calves	—	Healed
Gipstein *et al.*	1976	7	M	18	15 months post Tx HD	Buttocks, thighs, calves	4	Healed
		9	M	20	14 months post Tx	Trunk and limbs	3½	Died
Peterson	1978	3	?	?	2 years post Tx	Buttocks, thighs and lower legs	Subtotal	Died
		4	?	?	3 years post Tx	Buttocks, thighs and lower legs	—	Died
		5	?	?	Post Tx	Trunk, buttocks, thighs, scrotum, penis	—	?
Kossard & Winkelmann	1979	10	F	56	Uraemia	Lower abdomen, legs	—	Died
Perloff *et al.*	1979	1	F	20	2 years post Tx	Thighs	3½ & autotransplantation	Died
		2	M	30	2½ years post Tx	Legs	4 & autotransplantation	Died
		3	M	26	3 years post Tx, subtotal PTx and auto Tx 6 months post Tx	Trunk, buttocks, thighs, scrotum, penis	—	Died
Present paper	1983	1	F	49	2 months post Tx	Lower abdomen, thighs	3½	Died
		2	F	39	12 months post Tx, chronic rejection	Trunk, buttock, thighs	3½	Died

* PD = peritoneal dialysis; Tx = renal transplant, good graft function unless otherwise stated; HD = haemodialysis; PTx = parathyroidectomy; auto Tx = autotransplantation.

TABLE 2. Clinical data of patients with distal skin necrosis

Author	Year	Case No.	Sex	Age	Status*	Areas of skin involvement	No. of parathyroid glands removed	Outcome
Bryant & White	1898	1	M	6 months	Uraemic	Right foot	—	Died
Brown & Ginsburg	1940	1	F	55	Uraemic	Lower legs	—	Healed
Richards	1951	1	F	44	Uraemic	Toes	—	Died
Friedman *et al.*,	1969	1	M	29	PD	Fingers	—	Died
		2	F	52	Uraemic	Fingers	—	Amputation right hand
Rosen *et al.*	1972	1	M	60	Uraemic	Fingers, right heel, glans penis	—	Died
Hallgren *et al.*	1975	1	M	51	17 months post Tx	Toes	2½	Healed
		2	M	56	14 months post Tx	Toes, fingers	3	Died
Gipstein *et al.*	1976	1	F	41	3 months post TX	Fingers, ankles, toes	3½	Healed
		2	F	20	11 months post Tx	Lower legs	4	Healed
		3	F	53	HD	Fingers	4	Healed
		4	M	63	Uraemic	Right calf	3½	Healed
		5	M	41	HD	Fingers	3	Healed
		6	M	43	HD	Fingers	4	Died
		8	M	30	10 months post Tx	Fingers, left heel and toes	—	Amputation of fingers
		10	F	48	3 years post Tx	Right leg	3½	Healed
		11	F	39	HD	Right hand fingers	3½	Amputation of fingers
Blumberg & Weidmann	1977	1	M	63	HD	Lower legs	Subtotal	Healed
		2	M	52	HD	Lower legs	3	Healed
Peterson	1978	1	?	?	Post Tx	Toes	—	Died
		2	?	?	Post Tx	Toes	—	Amputation
Perloff *et al.*	1979	4	F	42	2 years post Tx	Calves, right ankle	Total & auto-transplantation	Healed
Collier & Mitch	1980	1	M	21	Uraemic	Fingers, toes	3½	Healed
Present paper	1983	3	F	43	9 years post Tx chronic rejection	Lower legs	—	Healed
		4	F	53	Uraemic	Lower legs	—	Died

* Abbreviations as in Table 1

necrosis involving the trunk, shoulders, buttocks or thighs (Table 1) the lesions covered large areas and spread rapidly. Nineteen of these patients died—ten out of eleven after parathyroidectomy and nine out of eleven without the 'benefit' of this operation.

Amongst twenty-five patients with distal skin necrosis involving the fingers of lower limbs beyond the mid-calf level (Table 2), digital gangrene was a frequent occurrence. However, while amputation of a digit or a limb was required in some instances, the disease appeared self-limiting and seventeen of these twenty-five patients survived (P value for difference in survival between 'distal' and 'proximal' groups: $< 0{\cdot}001$).

Parathyroidectomy was performed in fourteen patients with distal lesions. Two of the parathyroidectomized patients in this group died, compared to six of eleven unoperated patients. The difference between the survival rates of parathyroidectomized and non-parathyroidectomized patients was not statistically significant.

There were no histological differences between proximal and distal lesions either in our patients or in patients described in the literature. The factors governing the distribution and the spread of skin necrosis in these patients remain obscure. A spatial association appears established between skin necrosis and vascular lesions which are clearly different from both intimal atheroma and medial calcification. The relationship between these lesions and renal disorders is unknown and the role of parathyroidectomy remains unproved.

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Focal acral hyperkeratosis

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SUMMARY

We report fifteen cases showing focal acral hyperkeratosis. They were clinically identical to acrokeratoelastoidosis (AKE) but lacked the elastorrhexis which is a feature of AKE. The name focal acral hyperkeratosis is proposed for this condition which differs histologically from AKE.

Costa (1952, 1953, 1954a, b) described a clinical entity which he called acrokeratoelastosis lichenoides, though he subsequently came to prefer the term acrokeratoelastoidosis. Several cases clinically and histologically identical have since been reported by Poiares Baptista (1963) Bessière (1974), Haneke, Schwarzenbach & Hornstein (1977) and Jung *et al.* (1974) who comprehensively reviewed the condition.

Fifteen patients have now been reviewed by us. All have lesions clinically identical to those described in Costa's reports. With the exception of one patient who is Arab all patients are Negroes; three are male and twelve are female, the age range being 2–38 years. In only two patients was the onset of the disease noticed after the age of 20 years. In nine patients the onset was noticed before the age of 10 years and in the remaining six patients the lesions were first noticed in the teenage years. All patients had typical oval or polygonal crateriform papules along the borders of the hands and feet (Figs 1 & 2) with involvement of the junctional skin of the interdigital cleft in three patients and involvement of the wrist creases in two patients. Seven patients showed thickening and hyperpigmentation associated with hyperkeratotic papules over several of the interphalangeal joints of the fingers (Fig. 3) and in five patients similar changes were to be seen over the metacarpophalangeal joints (Fig. 4). Five patients showed marked callus-like lesions over the interphalangeal joints of the toes (Fig. 5) and two patients in addition showed discrete hyperkeratotic lesions over the heels. In six patients the condition was familial (a father and three daughters, and a mother and daughter). One other patient had a family history of the disease but the relatives were not available for examination. In the remaining twelve patients there was no family history of the condition even when the number in the family was large. One of the three sisters in this series exhibited nail dystrophy of several finger and toe nails; this was said to have followed trauma some years previously.

Histological examination of papular lesions has revealed identical changes in all of fourteen patients in whom it was performed. Biopsy was not performed on the father of the three sisters.

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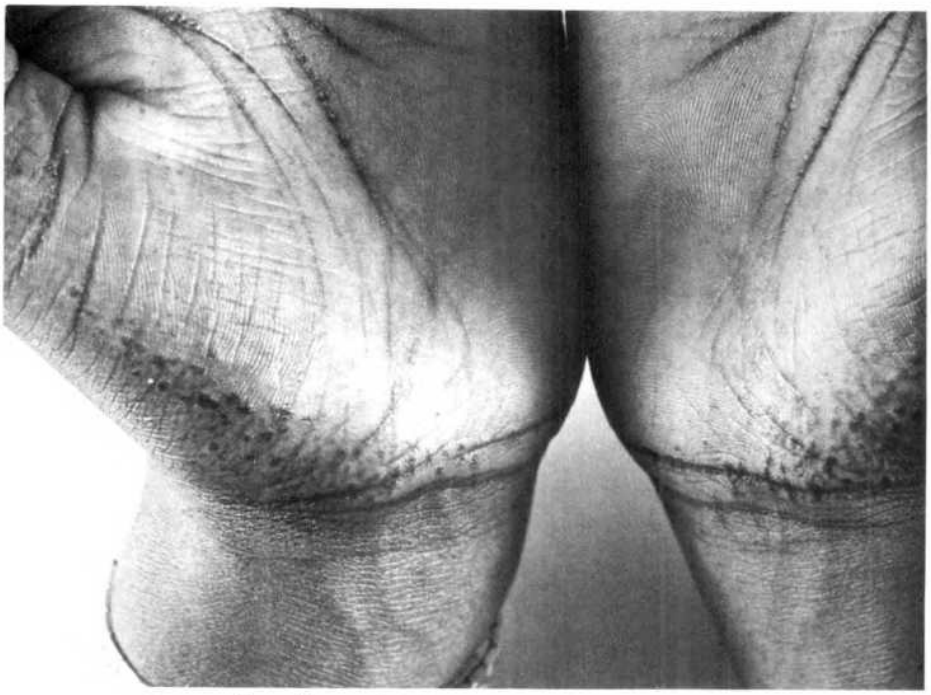


FIGURE 1. Crateriform and oval papules along the border of the hand.

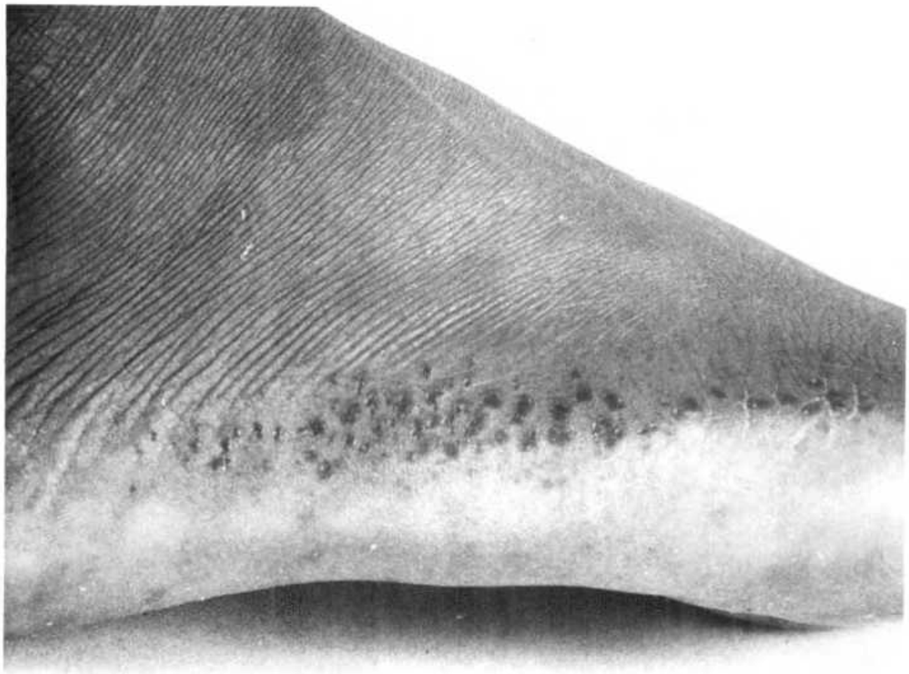


FIGURE 2. Oval and polygonal papules along the line of transgredience of the foot.

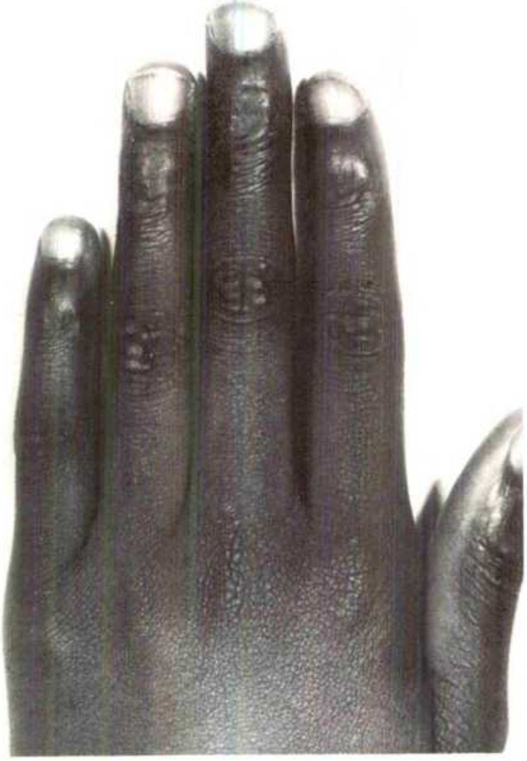


FIGURE 3. Hyperkeratotic papules within thickened hyperpigmented skin over the interphalangeal joints of the fingers and loss of cutaneous markings with hyperpigmentation over the terminal phalanges.

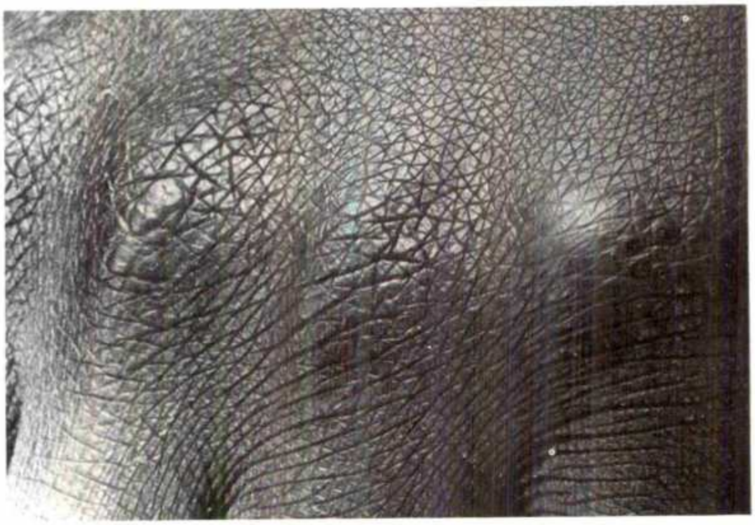


FIGURE 4. Hyperkeratotic papules over the metacarpophalangeal joints.

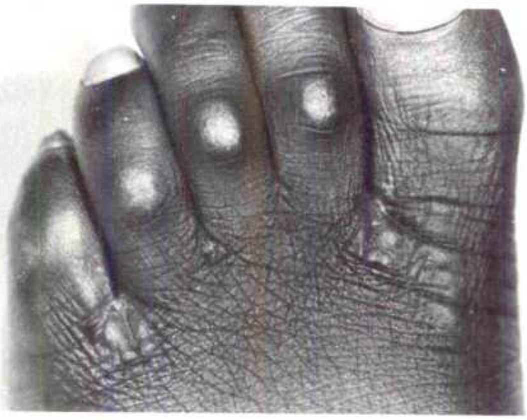
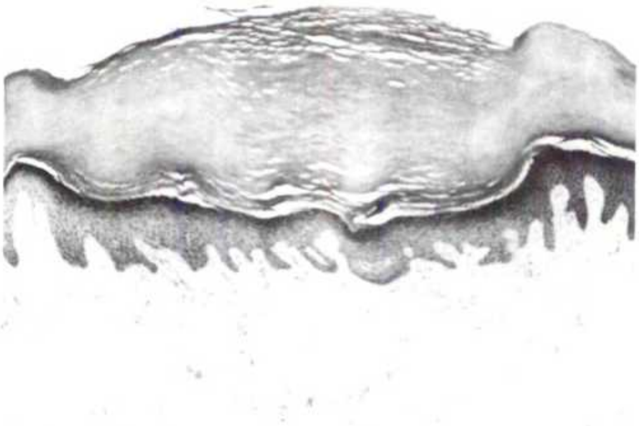


FIGURE 5. Callus-like lesions over the interphalangeal joints of the toes.



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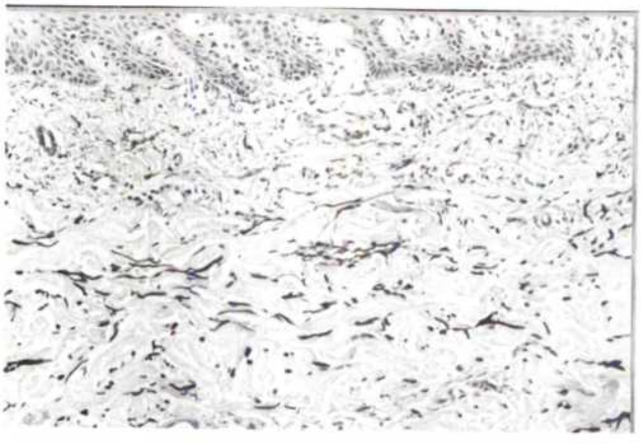

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FIGURES 6 & 7. Histology of a typical crateriform papule from the line of transgredience of the hand to show hyperkeratosis (Fig. 6) and the lack of elastorrhexis (Fig. 7).

Focal hyperkeratosis was found in association with a clavus-like depression in the epidermis which was acanthotic (Fig. 6). In five patients there was mild dilatation of the upper dermal capillaries, but in no patient was there any evidence of inflammation in the dermis. The collagen appeared normal and elastic stains showed the amount of elastin to be normal; in no patient was there any evidence of fragmentation of elastin. Histology of a biopsy of one of the dystrophic nails and underlying nail bed showed a rather atrophic Malpighian layer together with some hyperkeratosis and a horizontal fibroplasia of the dermis consistent with scar formation. Transmission electron microscopy revealed normal collagen and elastic tissue in five patients in whom this was performed. Fibroblast cultures from hyperkeratotic papules from the dorsa of the fingers of the three sisters in the series revealed no abnormality of collagen formation and the ratio of type I to type III collagen was normal.

We have also reviewed an additional six patients with a clinically identical disorder who were unwilling to be subjected to a skin biopsy. We have excluded these from the series.

DISCUSSION

The fifteen patients here described are clinically identical to patients described by Costa (1952, 1953, 1954a, b), Poiares Baptista (1963), Bessière (1974), Haneke *et al.* (1977) and more recently Highet, Rook & Anderson (1982). Characteristically the process begins in childhood or adolescence. The age range at presentation in our series is from 2 to 38 years, only two patients developing lesions for the first time after the age of 20 years. The majority of patients presented in the second or third decade of life. Six patients were members of two families (a father and three daughters, and a mother and daughter). One patient had a family history of the disease but the relatives were not available for examination. In the remaining eight patients the disorder seemed to have arisen sporadically. This distribution of familial and sporadic cases with an apparent autosomal dominant inheritance in the familial cases is in accordance with the reports of Costa (1952, 1953) and Jung *et al.* (1974).

The form and distribution of the lesions with a preponderance of crateriform or umbilicated papules along the lines of transgredience, i.e. the junction between the dorsal and palmar or plantar skin and their gradually increasing number over several years is also characteristic.

Our patients showed the histological hallmarks of acanthosis and marked focal hyperkeratosis, but in none of the fourteen patients in whom histology was performed was there any evidence of alteration of the collagen or paucity or fragmentation of the dermal elastic fibres in the reticular dermis. In five patients transmission electron microscopy revealed no abnormality of collagen or elastic fibres and the fibroblasts appeared normal. There was no evidence of the dense granules near the plasma membrane of the fibroblast reported in this condition by Massé *et al.* (1977). None of our patients showed clinical or histological evidence of solar degenerative change in the skin nor did they have hyperhidrosis, a clinical feature believed to be an associated feature of this condition (Costa, 1952, 1953). The history of preceding trauma to the dystrophic nails accompanied by histological changes in a nail biopsy consistent with scarring would suggest that the nail dystrophy occurring in one of the patients we report is unrelated to the clinical syndrome of focal acral hyperkeratosis.

Highet *et al.* (1982) divided acrokeratoelastoidosis into two clinically somewhat similar syndromes.

(1) An idiopathic, sometimes familial form without associated actinic change with onset usually in youth, i.e. the clinical form described by Costa.

(2) A clinically similar form comprising lesions developing later in life in patients with existing actinic damage and in whom repeated trauma may also be important. These lesions have been described by Burks, Wise & Clark (1960), Ritchie & Williams (1966), Kocsard (1964), Pomposiello & Mosta (1957) and Ramos E Silva (1967) and were given the name degenerative collagenous plaques of the hands by Burks, Wise & Clark (1960).

We consider this division misleading and that degenerative collagenous plaques of the hands form an entirely separate and distinct disorder. It is characteristically seen in white Caucasian males who have experienced years of sun exposure in manual outdoor occupations. The plaques are bandlike and extend from the tip of the thumb around the web to the radial side of the index finger. There is no foot involvement nor extensor surface hyperpigmentation. Histology does not show the neat disc of hyperkeratosis within a depression in the epidermis seen so clearly in acrokeratoelastoidosis. There is no familial predisposition.

Highet *et al.* also comment on the occurrence of a third group of patients sharing some clinical features with acrokeratoelastoidosis (AKE) but being histologically distinct. These are the patients reported by Matthews & Harman (1977) who had palmoplantar keratoderma in association with isolated hyperkeratotic papules on the backs of the fingers and the lateral margins of the hands, and in whom the elastic content of the dermis was normal. These patients are not included in the series we now report. However, the patient with lesions clinically similar to those of familial AKE but also without histological evidence of collagen or elastic tissue degeneration, and the four other similar Negro patients previously described by Matthews & Harman (1974), are included in this series.

We agree with the suggestion of Highet *et al.* that the term acrokeratoelastoidosis should not be applied to cases lacking the characteristic elastic degeneration of Costa's original description. It is for this reason that the first case to be recorded in the United Kingdom was described as 'AKE without elastorrhexis'—the clinical features being identical with his description but with no elastic alteration to be found. Costa has stated (personal communication to R.R.M.Harman) that in clinical AKE it may be necessary to cut many sections before elastoidosis is found, but in our extensive material on histological investigation, electron microscopy and fibroblast culture no abnormality whatsoever of collagen or elastic tissue can be discovered.

We, therefore, propose that the term AKE should be reserved for those cases showing the clinical features Costa described and exhibiting elastorrhexis histologically. The clinically similar but histologically dissimilar cases we report, we suggest, should be described under the title focal acral hyperkeratosis which in our experience is a much more frequently encountered disorder than acrokeratoelastoidosis. It appears to be a racially limited focal disorder of keratinization and pigmentation of insidious onset in childhood, reaching a maximum in early adult life and causing only cosmetic embarrassment. We have not found any form of treatment effective.

Degenerative collagenous plaques of the hands is an entirely separate condition occurring in an older age group of white Caucasians occurring only on the hands and related to sun exposure and trauma.

ACKNOWLEDGMENTS

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Is Dowling–Degos disease the same disease as Kitamura's reticulate acropigmentation?

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SUMMARY

A patient is described with the clinical and histopathological features of both Dowling–Degos disease and Kitamura's acropigmentatio reticularis. The possible identity of the two genodermatoses is discussed on the basis of the similarity of their clinical and histopathological features and of the presence of the two diseases in the family of the propositus.

The reticulate pigmented anomaly of the flexures, or Dowling–Degos disease (DDD), and the acropigmentatio reticularis of Kitamura (ARK) are two distinct genetic disorders of pigmentation.

We have observed a patient who had the clinical and histopathological features of both conditions.

CASE REPORT

A 52-year-old woman of Sicilian origin presented with a pigmentary disorder dating back to her early thirties. She reported that it had been preceded by a transitory erythematous stage, and that the macules had appeared in all regions of her body simultaneously.

On examination, a reticulate network of brownish macules was observed involving the nape, the lateral aspects of the neck, the presternal and the inter- and infra-mammary areas, the axillae, the lower abdomen, the groins and the inner thighs, the forearm, the dorsum of the hands and feet, and the shins and ankles (Figs 1–4).

Nails and hair were normal as well as the ocular and oral mucosae. Some breaks in the epidermal ridge pattern of the palms were seen (Fig. 5).

The 37-year-old sister of the patient presented with a 6-year history of a similar reticulate pigmentation involving only the acral areas. Her mother was reported to have had a reticulate pigmentation confined to the flexures. Their father was reported to be normal.

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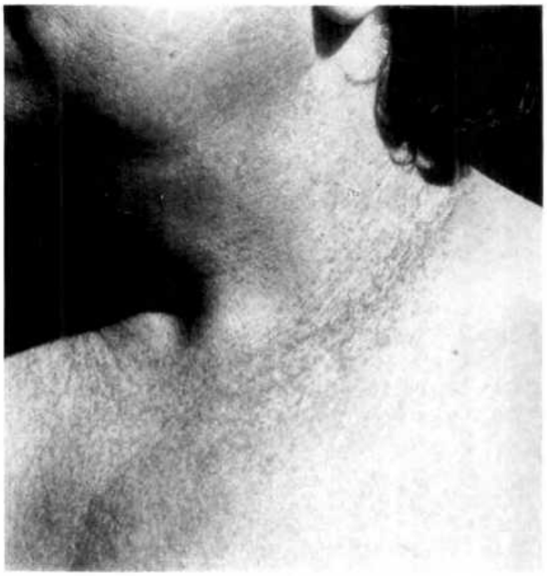

FIGURE 1. Pigmentation of the neck.

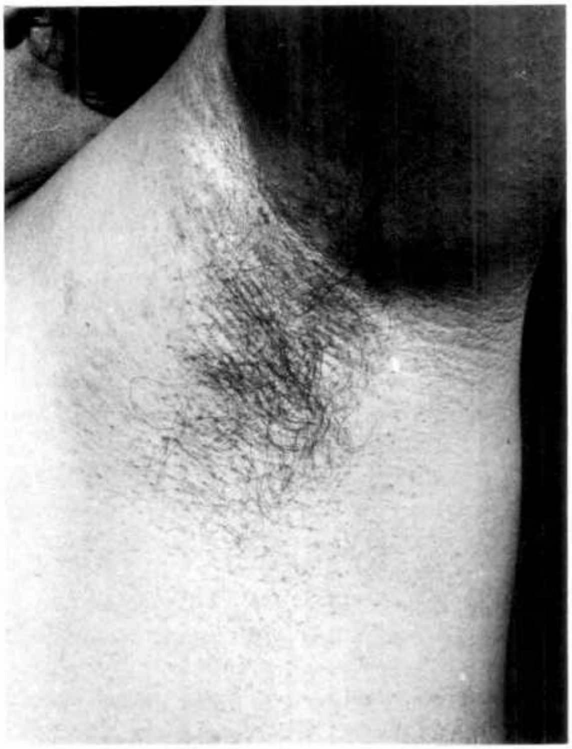

FIGURE 2. Macules of the axilla.

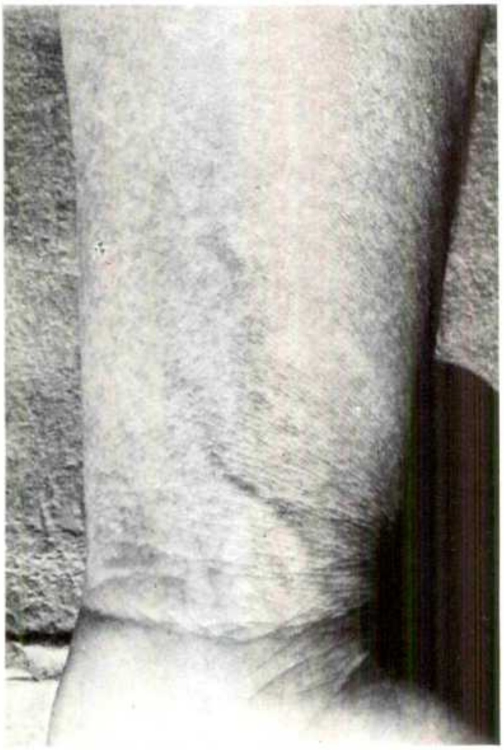

FIGURE 3. Pigmentation of the forearm.

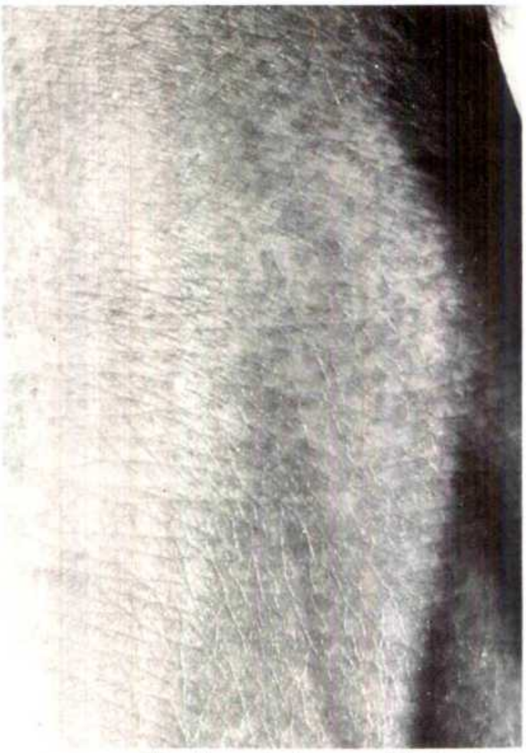

FIGURE 4. Macules on the dorsum of the hand.

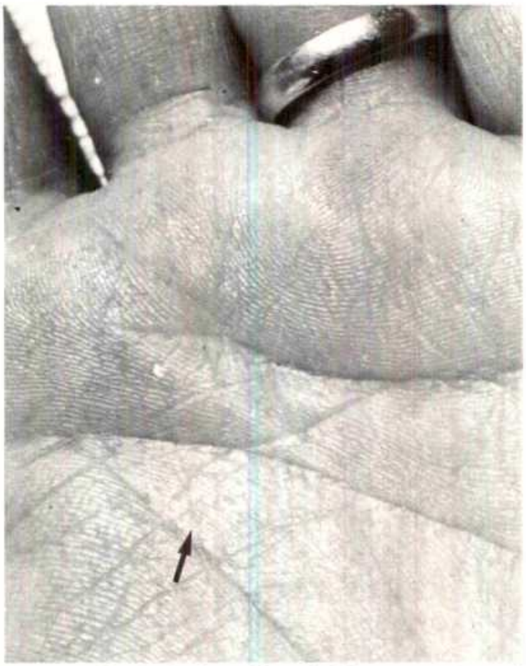

FIGURE 5. Breaks in the epidermal ridge pattern (arrow).

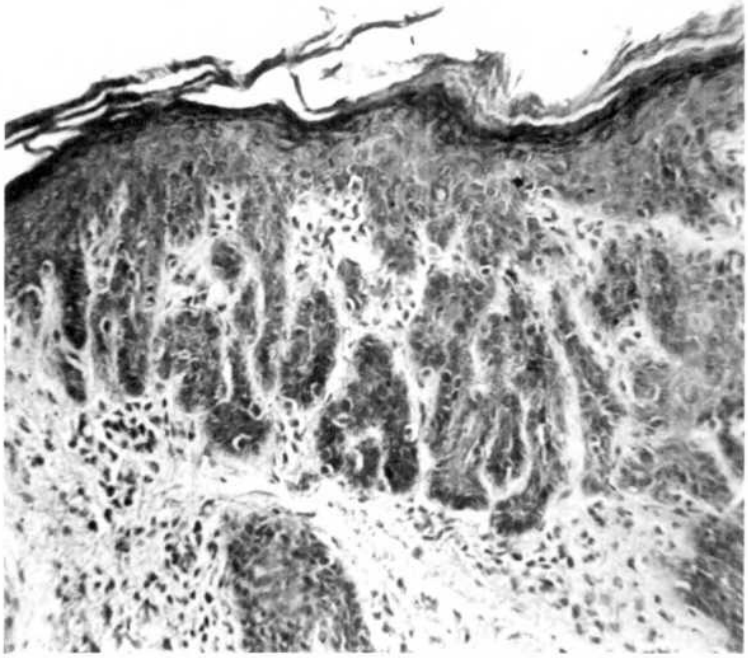

FIGURE 6. Typical histopathology of DDD of axillary lesions.

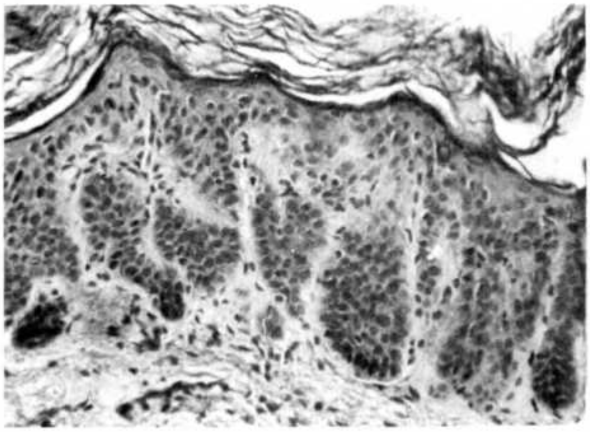

FIGURE 7. Typical histopathology of ARK on the dorsum of the hand. Note the slight atrophy of the epidermis above the elongated and pigmented rete pegs.

Biopsy specimens were taken from both the right axilla and the dorsum of the left hand of the proband. Both specimens showed similar features consisting of a delicate, hyperpigmented proliferation of the rete ridges, that was more prominent in the flexures (Figs 6 and 7), where it affected also the hair follicles. A slight atrophy of the epidermis was seen in the hand. No pigmentary incontinence was visible.

DISCUSSION

DDD (Dowling & Freudenthal, 1938; Degos & Ossipowski, 1954) is an autosomal dominant condition characterized by a reticulate brownish pigmentation of the flexures. The disorder mainly affects women and has a late onset. Histopathology reveals delicate, digitate and hyperpigmented downgrowths of epidermis and adnexae.

ARK (Kitamura & Akamatsu, 1943) is an autosomal dominant dermatosis beginning in the teens and displaying a reticulate, brownish and slightly depressed pigmentation of the extremities. Palmar pits or breaks in the epidermal ridge pattern are another typical feature. Histopathology exhibits a slight atrophy of epidermis and delicate, elongated and occasionally ramified rete pegs which, particularly on their apex, contain deposits of melanin.

The main differences between the two conditions are:

(1) The acral location of macules in ARK and the flexural involvement in DDD.
(2) A slight depression in the ARK macules whereas DDD macules tend to be papular.
(3) The palmar pits in ARK.
(4) The epidermal atrophy in ARK histopathology.
(5) The early onset in ARK and the delayed onset in DDD.

Our patient shows the clinical and histopathological characteristics of both conditions. The pigmentation of the nape, neck, chest and flexures had the distinctive clinical and histopathological features of DDD, whereas the lesions of the extremities were clinically very suggestive of ARK, macules being associated with palmar pits. We were not able to find any depression in the macules and the age of onset was rather late, but no alternative clinical diagnosis could be made. There was not the mixture of hyper- and hypo-pigmented macules of Dohi and Komaya's acropigmentation, nor were there the changes of nails, teeth and mucosae of dyskeratosis congenita, nor was there the pigmentary incontinence of dermopathia pigmentosa reticularis (Griffiths, 1976). Finally, the histopathology was definitely that of ARK (Kitamura, Akamatsu & Kirokawa, 1953).

Three possibilities can be considered. Is our patient a unique case of DDD extending to the extremities, or an extraordinary example of coexistence of two exceedingly rare genodermatoses, or are the two conditions the same disease?

We favour the last hypothesis. In fact, the reticulate macules of DDD and ARK are very similar, leaving aside depressions and palmar pits which, however, are not a constant feature (five of the seven cases of Griffiths, 1976). The histopathology is strikingly similar in the two conditions. The digitate down-growths of DDD are more accentuated, but this is not surprising bearing in mind the tendency of the flexural epidermis to vegetate in many pathological conditions.

DDD has surprisingly never been taken into diagnostic consideration in ARK literature and, in fact, only recently was it called to attention by the excellent review of Wilson-Jones & Grice (1978). It may be that the authors of reports on ARK did not envisage this diagnostic possibility.

Finally, our patient appears to belong to a family in which DDD and ARK are curiously distributed. In the light of the autosomal dominant transmission of DDD (Brown, 1982; Crovato, Nazzari & Rebora, 1983), the mother of our patient should be expected to have produced children with DDD, but in fact the propositus showed both DDD and ARK and her sister showed only ARK. The sole explanation is that the two conditions are different phenotypic expressions of the same genodermatosis.

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Generalized pustular psoriasis precipitated by phenylbutazone and oxyphenbutazone

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SUMMARY

We describe two patients who developed generalized pustular psoriasis after taking phenylbutazone and oxyphenbutazone respectively, and discuss the possible pharmacological mechanism of this effect.

Generalized pustular psoriasis is a rare and serious form of the disease which has been recognized to occur in association with certain drug therapies, particularly systemic and topical corticosteroids (Baker & Ryan, 1968; Baker, 1976), lithium (Lowe, 1978), and beta blockers.

We report two patients who developed generalized pustular psoriasis after treatment with phenylbutazone and oxyphenbutazone. The second patient was unfortunate enough to undergo an inadvertent therapeutic challenge.

Case 1

A 39-year-old woman who had suffered from plaque psoriasis since the age of 5 received phenylbutazone in November 1979 for superficial thrombophlebitis of the right leg. Her psoriasis had previously been satisfactorily controlled with coal tar, dithranol and topical steroid preparations. The patient was using dithranol pomade and coal tar extract, allantoin and hydrocortisone (Alphosyl® HC) when phenylbutazone was administered.

Within 10 days the patient was admitted as an emergency with generalized pustular psoriasis involving most of the trunk and limbs. She was toxic with a leucocytosis of $15{\cdot}8 \times 10^9/\text{l}$. As well as topical therapy, ACTH injections and later oral triamcinolone were required to control pustulation.

After 10 days plaque type psoriasis emerged and within 3 weeks she was discharged home having discontinued triamcinolone and requiring only topical therapy with coal tar and salicylic acid ointment (B.P.C.).

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Case 2

A 78-year-old lady was admitted with generalized pustular psoriasis in December 1980 after taking three doses of oxyphenbutazone (Tandecote®) for pain in the shoulder following a fracture. There was a history of a similar eruption with phenylbutazone in 1973 but she had been free of skin disease subsequently.

Pustulation had started on the buttocks and later became generalized. The patient was dehydrated, febrile (38°C) and had an intense neutrophil leucocytosis, (w.c.c. $26{\cdot}4 \times 10^9$/litre, 96% neutrophils). Intensive treatment was required including oral prednisolone 30 mg per day. Control was not achieved with systemic steroids alone and azathioprine 150 mg per day was added. Subsequently it was possible to reduce the systemic therapy.

DISCUSSION

The close time relationship suggests that the administration of phenylbutazone and oxyphenbutazone was responsible for precipitating generalized pustular psoriasis in these patients.

Phenylbutazone and its related metabolite oxyphenbutazone commonly cause adverse effects and the risk of agranulocytosis limits their use as anti-inflammatory agents.

The non-steroidal anti-inflammatory drugs are believed to exert their effects by inhibition of prostaglandin synthetase, thus reducing the production of prostaglandins (Vane, 1971). Prostaglandins are now known to be important modulators of adenyl cyclase in mammalian cells and their effects on cyclic AMP levels in mammalian epidermis have been demonstrated (Adachi *et al.*, 1975). Studies have shown that adenyl cyclase activity is reduced in psoriatic plaques and that it can be stimulated by prostaglandin E_2 (Mui, Hsia & Halprin, 1975). Cyclic AMP has important roles in cellular proliferation and maturation, and it seems likely that decreased cyclic AMP levels are important in the abnormal epidermal activity of psoriasis (Voorhees *et al.*, 1973). Thus there is a pathophysiological mechanism to explain the worsening of psoriasis by inhibitors of prostaglandin synthetase leading to reduced production of prostaglandins and cyclic AMP.

It is interesting that an exacerbation of psoriasis by indomethacin has recently been reported (Katayama & Kawada, 1981). This drug is also an inhibitor of prostaglandin synthetase.

There is an alternative explanation for the occurrence of the uncommon pustular form of psoriasis after the administration of the prostaglandin synthetase inhibitors. It is possible that by the inhibition of prostaglandin synthetase, more of the prostaglandin precursor arachidonic acid becomes available for lipoxygenase pathway metabolism. This pathway leads to the production of potent neutrophil chemotactic agents such as leukotriene B_4 and 12-hydroxyeicosatetraenoic acid. Indeed recent experimental evidence suggests that phenylbutazone can potentiate leukocyte migration at low doses which have no significant effect on prostaglandin production (Eakins *et al.*, 1980). Neutrophil invasion of the epidermis is a prominent feature of pustular psoriasis.

Whatever the true mechanism, the occurrence of episodes of generalized pustular psoriasis in previously stable psoriatics suggests that these drugs should be used with special caution in this group of patients.

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Comment

NEUTROPHIL POLYMORPHONUCLEARS, MEDIATORS AND THE PATHOGENESIS OF PSORIASIS

The role of the neutrophil polymorphonuclear leukocyte in the pathogenesis of the psoriatic lesion is the subject of sustained interest. The presence of neutrophils within the acanthotic epidermis is a familiar histological feature of psoriasis, but the relationship of neutrophils to the histogenesis of psoriasis has been actively debated. Pinkus & Mehregan (1966) proposed that neutrophils gained access to the epidermis through the intermittent action of a 'squirting papilla', increase in epidermal mitotic activity being a consequence of this process. Subsequent attempts to confirm the concept of neutrophil immigration into epidermis as the prime histological event in the pathogenesis of psoriasis have been bedevilled by imprecise definitions of the 'earliest' lesion of psoriasis. Ragaz & Ackerman (1979) in a review of 300 biopsy samples (twenty from barely visible pinhead-size macules devoid of scales) observed early exocytosis of lymphocytes and histiocytes. However, the apparently abrupt appearance of neutrophil spongiform pustules in later papular lesions is puzzling and unexplained. Jablonska's studies of 'pre-pinpoint papules' in developing Koebner reactions produced by sellotape stripping revealed exocytosis of neutrophils into the epidermis as the earliest cellular change (Jablonska *et al.*, 1982). This method can be criticized on the grounds that a papule is not necessarily the earliest stage in evolution of a psoriatic lesion. A more dynamic approach is clearly needed—perhaps an adaptation of the skin chamber method of Dubertret, Lebreton & Touraine (1982) to allow its use in the evolving psoriatic lesion.

Notwithstanding these issues, neutrophil invasion of epidermis is a prominent event in psoriasis which serves to shift attention away from epidermal cell kinetics and growth control mechanisms and to strengthen mounting evidence of the importance of dermo-epidermal interaction in the genesis of psoriatic lesions. Because neutrophils are not only highly reactive to a wide range of autocoids, but are also themselves a Pandora's box of pharmacological activities, the molecular basis of neutrophil behaviour in psoriatic skin has begun to attract pharmacological attention. Examination of pharmacological reactivity of peripheral blood neutrophils to chemotactic and neutrophil-degranulating substances is an unsatisfactory approach and in practice has yielded confusing results (Breathnach, Carrington & Black, 1981; Glinski *et al.*, 1978; Wahba *et al.*, 1979; Tagami & Ofuji, 1976; Kruegar, Hill & Jederberg, 1978; Silny *et al.*, 1980; Bloomfield & Young, 1983; Preissner, Schroder & Christophers, 1983). Sampling error may be partly to blame. Peripheral blood neutrophils are a subpopulation, not necessarily representative of the majority which have sequestered in the bone marrow and other tissues, including psoriatic skin. Examination of function of neutrophils *in situ* in involved or uninvolved skin may prove a better approach (Breathnach *et al.*, 1981; Dubertret *et al.*, 1982).

The balance of histological evidence suggests a key role for neutrophils in the pathogenesis of psoriasis, and also poses two important questions. What is the nature of the chemoattractant activity which causes accumulation of neutrophils in the epidermis? How does the presence of activated neutrophils in the epidermis influence keratinocyte growth and differentiation?

A number of authors have demonstrated leukocytic chemotactic activity in psoriasis scale (Langhof & Muller, 1966; Tagami & Ofuji, 1976, 1977). Tagami and Ofuji prepared aqueous

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extracts from skin scale of psoriasis and other exfoliative disorders. Extracts were examined for chemotactic activity by the Boyden Chamber method, using human leukocyte indicator cells. Only extracts from psoriasis scale showed significant chemoattractant properties. Most of this activity eluted in the 12,000 molecular weight region on Sephadex G-200 chromatography. Antiserum inhibition studies suggested to the authors that C3a might be a major constituent, but since C3a is not significantly chemoattractant, it is likely that the antiserum used lacked specificity. Lazarus, Yost & Thomas (1977) reported that psoriatic plaque contained a complement-dependent chemoattractant factor. This factor, which was not found in the scale of pityriasis rubra pilaris, was inhibited by diisopropyl fluorophosphate, suggesting that it is a product of a protease, subsequently identified as cathepsin I (Hatcher, Lazarus & Levine, 1977). Lazarus' findings suggest that chemoattractant polypeptides, as yet unidentified, provide the signal for migration of neutrophils into the epidermis. That chemoattractant activity in epidermis is mainly attributable to complement components is proposed by Jablonska *et al.* (1975). IgG stratum corneum antibody, bound to stratum corneum antigen, leads to local complement activation and release of chemotactic complement components. Since the antibodies occur in psoriatics and non-psoriatics alike, and since similar epidermal deposits are found in other inflammatory skin disorders, the presence of complement components may be secondary rather than primary.

A recently described product of arachidonic acid metabolism, leukotriene B4 (Borgeat & Samuelsson, 1979) which is at least as potent a leukocyte chemoattractant as C5a (Ford-Hutchinson *et al.*, 1980; Camp *et al.*, 1982) has now been shown to be present in increased amounts in the involved skin of psoriasis. Using a skin superfusion technique to recover exudate from involved epidermis of psoriasis, Brain *et al.* (1982a) have shown that exudate from psoriatic plaque contains polar acidic lipid chemokinetic activity which is revealed on straight-phase high performance liquid chromatography to consist of two distinct fractions. One of these co-chromatographs with mono-hydroxy-eicosatetraenoic acids (mono-hetes). These compounds, which are products of the action of lipoxygenases on arachidonic acid, include several chemoattractant agents (Turner *et al.*, 1975). Subsequent analysis by gas chromatography–mass spectrometry has confirmed the presence of LTB4 in this fraction (Brain *et al.*, 1983). At least two possible sources of the LTB4 activity have been considered. Psoriatic epidermis contains significant numbers of neutrophil polymorphonuclears, which in response to activation, synthesize LTB4 (Borgeat & Samuelsson, 1979). Keratinocytes may also contribute. Brain *et al.* have shown that if cultured human keratinocytes are incubated with a calcium ionophore, chemokinetic activity co-chromatographing with LTB4 can be recovered from the culture supernatant (Brain *et al.*, 1982b), although the yield is low compared with polymorphonuclears. It can therefore by hypothesized that minor skin trauma causes formation of LTB4 by keratinocytes leading to exocytosis of neutrophils into the epidermis. Activated neutrophils could form more LTB4, evoke further neutrophil emigration and thus amplify the process. Subsequent steps are also speculative. Keratinocytes of psoriatic subjects are genetically programmed to respond to an appropriate signal by increased division and disordered differentiation. Possible signals include a soluble factor derived from intraepidermal neutrophils, perhaps a proteinase as suggested by Lazarus & Gilgor (1979).

The biosynthesis of the leukotrienes is catalysed by the cytoplasmic enzyme 5-lipoxygenase. Suppression of the activity of this enzyme in skin might be expected to cause remission of psoriasis. Benoxaprofen is a non-steroid anti-inflammatory drug with a selective inhibitory action on lipoxygenase (Cashin, Dawson & Kitchen, 1977). It was introduced as an anti-arthritis drug, but was soon withdrawn owing to a number of instances of severe toxicity. Prior to its withdrawal, preliminary studies of its efficacy in psoriasis vulgaris gave encouraging results (Allen & Littlewood, 1982). It is to be hoped that further opportunities will arise for fully controlled therapeutic studies of lipoxygenase inhibitors in psoriasis.

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Obituary

Dr P.W.Grant

Dr Peter Grant, dermatological specialist with the Highland Health Board, died on 9 February 1983, aged 59, after a short illness. Peter Warwick Grant was born in Glasgow in 1924, had his schooling at Glasgow Academy and his medical training at Anderson and St Mungo's Colleges in Glasgow, where he qualified in 1948. After junior medical appointments in Glasgow, and a spell of National Service from 1950–52, Dr Grant specialized in dermatology and had his training in Derby, Edinburgh and Glasgow.

In 1965 he was appointed Medical Assistant in Dermatology to the Highland Health Board, where in conjunction with Dr Norman McKinnon, he established a very comprehensive dermatological service in a region which extended from Nairn in the East to Fort William in the West, from Inverness to Caithness, and included the Hebridean Islands of Skye and Lewis.

Dr Grant was very well known in British dermatological circles. In debate he was direct and at times outspoken, quick in action and determined by nature. At clinical meetings he spoke with knowledge, wit and with an authority that few would challenge. As a clinician he was invariably friendly and kindly; his life and work will long be remembered by patients and staffs, who held him in the highest regard. He was a most likeable, reliable and loyal colleague, whose word was his bond, and whose greatest pleasure was in a task well done.

His writings include papers on juvenile dermatitis herpetiformis, napkin dermatitis, do's and don'ts in dermatology and 'A singular dermatosis in Mongols'. As a teacher he was painstaking in his preparatory work, and lucid in his exposition.

Apart from his clinical ability and skill Dr Grant had a flair for administration which was turned to account in the Highland area, in the British Medical Association and in the Scottish Dermatological Society, the present success of which owes a great deal to his advice and prompting over the years.

Peter's hobbies included golf, an appreciation of the opera and of classical music. Golf he played with panache and skill; in this field his administrative ability made him a much sought after committee member at Nairn, where he was recently elected as Captain. He was also Vice-President of the North District Association of the Scottish Golf Union.

Peter, in 1952, married Pat. His devotion to his family of six daughters and three sons is manifest in their happy home life, and in their impressive academic achievements. To Pat and her family we extend sincere sympathy in their grievous loss. They can surely, in future, recall with pride the memory of a husband and father whose life was given over to the service and care of others.

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British Journal of Dermatology (1983) **109,** 120–125.

Correspondence

Type C retrovirus-like particles in mycosis fungoides

SIR, There is currently much interest in the possible viral aetiology of certain human T-cell malignancies. Gallo has recently isolated a new type C retrovirus (human T-cell leukaemia-lymphoma virus or HTLV) from patients with mycosis fungoides (MF) and the Sézary syndrome (Poiesz *et al.*, 1980; 1981). HTLV is now considered to be identical or very similar to the adult T-cell leukaemia-lymphoma virus (ATLV), the possible causative agent of Japanese and other similar forms of T-cell leukaemia and lymphoma (Popovic *et al.*, 1982).

HTLV and ATLV particles have so far only been isolated from T cells maintained in culture. There has been only one previous report describing type C retrovirus-like particles in uncultured tissues from cases of cutaneous T-cell lymphoma (CTCL) (Van der Loo *et al.*, 1979). The particles were present within both the skin and lymph nodes but were absent from control tissues.

We describe two patients with infiltrated plaques of mycosis fungoides in whom axillary lymph node biopsy provided valuable information regarding the possible viral pathogenesis of the disease. Both patients had extensive cutaneous involvement but without any significant lymphadenopathy. Axillary lymph node biopsies were performed as part of a larger prospective study investigating the pathological staging of CTCL.

Light microscopy of the nodes from both patients showed florid so-called dermatopathic lymphadenitis. Large convoluted lymphocytes were not apparent and the appearances were not considered diagnostic of involvement by CTCL. Transmission electron microscopy showed that the sinus cells consisted largely of Langerhans cells identified by the presence of characteristic cytoplasmic organelles (Birbeck granules) and

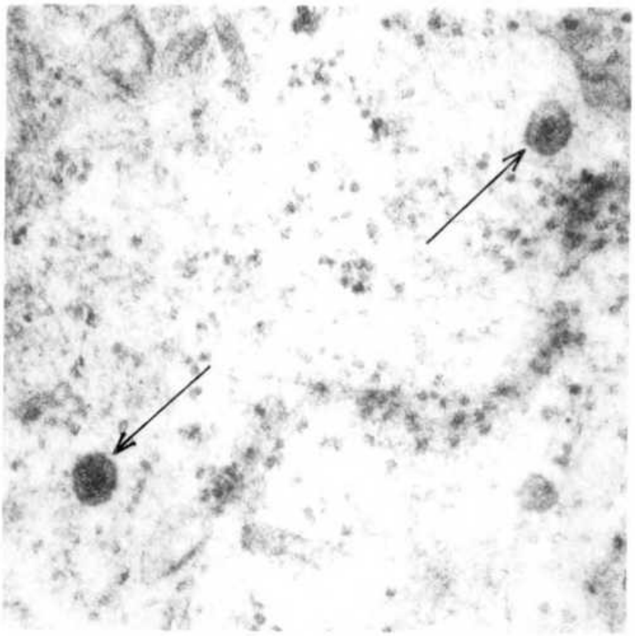

FIGURE 1. Type C retrovirus-like particles in the cytoplasm of a Langerhan's cell. (Transmission electron micrograph, × 60,000.)

interdigitating reticulum cells. In addition, mature type C retrovirus-like particles were identified within the cytoplasm of Langerhans cells from both of these patients. The particles measured 95–115 nm and were characterized by an electron-dense core surrounded by an outer membrane separated by a more electron-lucent region (Fig. 1). Extracellular particles and budding from the plasma membrane were not identified.

Immunoperoxidase studies with monoclonal antibodies for subpopulations of lymphocytes revealed that in one patient the T4:T8 ratio was less than 3. In comparison, the other patient showed an expansion of T4 (helper) lymphocytes with a T4:T8 ratio in excess of 10. It is of interest that the node from the latter patient showed relatively more Langerhans cells with larger numbers of Birbeck granules and retrovirus-like particles.

These findings confirm the observation by Van der Loo *et al.* (1979) that type C virus-like particles can be identified in the nodal Langerhans cells of patients with CTCL. We also feel that the work adds further support for the hypothesis that the initiating event in MF may be an infection of Langerhans cells by type C retrovirus (MacKie, 1981). It is also of immense importance that patients with MF clinically confined to the skin are found to possess significant abnormalities in lymph nodes when specialized techniques are used. This is in agreement with the view that CTCL in its early stages involves helper T-cell and Langerhans cell interactions in both the skin and lymph node (Edelson, 1980).

We now intend to investigate reverse transcriptase activity within the particles, as such activity was reported by Van der Loo (1979). However, it is also desirable that hybridization studies are performed to further characterize the virus and that ultrastructural immunolocalization is undertaken to ascertain whether the particles contain protein P 24, the main structural core antigen of HTLV. We also need to determine whether patients with retrovirus-like particles in their Langerhans cells elicit an antibody response against HTLV. A HTLV antibody response has already been reported in occasional patients with CTCL (Gallo, 1982). We hope that these questions will be answered in the near future.

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The relapse rate for rosacea after treatment with either oral tetracycline or metronidazole cream

SIR, 1% metronidazole cream is significantly better than a placebo cream in the treatment of rosacea and is as effective as oral oxytetracycline (Gamborg Nielson, 1983a,b). A follow-up study to assess the relapse rate after stopping treatment has now been performed.

I studied thirty-three consecutive rosacea patients (fourteen men, nineteen women; average age 48 years, range 29–75 years) who were treated with 1% metronidazole cream either once daily or once every second day, for 4 months. In addition I followed up two groups from my earlier studies (Gamborg Nielson, 1983a, b). The groups were thus as follows: (1) daily treatment with 1% metronidazole cream for 4 months, (2) treatment every second day with 1% metronidazole cream for 4 months, (3) daily treatment with 1% metronidazole cream for 2 months and (4) treatment with 250 mg oxytetracycline taken orally twice a day for 2 months. The number of relapses in each group 6 months after conclusion of treatment was as follows, the previously described criteria (clinical, photographic and patient's opinion) being used for assessment.

TABLE 1.

Group	Number of patients	No relapse	Relapsed at 0–3 months	Relapsed at 3–6 months	*P* value using chi-square test
1.	16	9 (56·3%)	3 (18·8%)	4 (25·0%)	$P<0{\cdot}05$
2.	17	8 (47·1%)	1 (5·9%)	8 (47·1%)	$P<0{\cdot}025$
3.	23	7 (30·4%)	4 (17·4%)	12 (52·2%)	Not significant
4.	23	3 (13·1%)	11 (47·8%)	9 (39·1%)	

There were significantly fewer relapses in groups 1 and 2 compared with group 4, but there was no significant difference between the relapse rate in groups 3 and 4.

These results suggest that metronidazole cream is at least as effective as oral tetracycline in preventing relapses.

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Mandibular resorption in systemic sclerosis, and its possible association with Scl-70 antibody

SIR, We read with great interest the recent case report of mandibular resorption in systemic sclerosis (Ryatt *et al.*, 1982).

Recently, we found three such cases out of eighteen confirmed patients with systemic sclerosis and reported our experience (Sato *et al.*, 1982). In brief, all of our three cases showed the resorption of both angles of the mandible and each one showed coronoid resorption as well. Though the aetiology of the bone resorption is unknown, we found Scl-70 antibody in all three sera of those patients having bone changes. Scl-70 antibody is a recently recognized anti-ENA (extractable nuclear antigen) antibody which relates to sclerotic changes (Douvras, Achten & Tan, 1979). Since we found this antibody in only four of eighteen systemic sclerosis sera, including those of the three patients having bone changes, we speculate that Scl-70 antibody may play a role in producing bone changes of the mandible in systemic sclerosis patients.

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Retinoids and the gastric mucosa

SIR, Patty *et al.* (1982) have reported a beneficial effect of vitamin A on gastric ulceration. The synthetic aromatic retinoids may have a similar action on gastric mucosa as illustrated by the following patient.

A 55-year-old man had experienced for 2 years severe erosive gastritis which was resistant to several treatments including cimetidine and carbenoxolone. He had coincidentally palmoplantar psoriasis and this was treated with etretinate (Tigason®) 75 mg daily. The psoriasis responded satisfactorily, and within 6 months of etretinate treatment both his dyspeptic symptoms and the gastroscopic appearances of the gastric mucosa had dramatically improved.

The aromatic retinoids have been used in a variety of disorders of cutaneous keratinization, but they may also have beneficial effects on mucous membranes (Elias & Williams, 1981), and the present report indicates that they may influence the gastric mucosa in a similar manner to vitamin A. However, although retinoids may theoretically protect against the development of gastric carcinoma (Fujimaki, 1926) their ability to protect against the development of lymphoma (Abb & Deinhardt, 1981) is open to question, since the present patient's general condition deteriorated after a further three months of treatment and he was found to have disseminated Hodgkin's disease.

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Isotretinoin (13cis-retinoic acid) and serum lipids

SIR, We read with interest the papers by Lyons *et al.* (1982) and Marsden, Shuster & Laker (1983) regarding the isotretinoin-associated changes in serum lipids. We should like to make the following comments.

First, it is recognized that certain dietary manipulations such as a high carbohydrate diet (Stone & Connor, 1962) may cause transient changes in serum lipids which tend to reverse despite continuation of the diet. Since isotretinoin is a very effective treatment for acne, it would be important to know whether the changes in serum lipids were transient. Therefore the examination of serum lipids after a longer period, for example 1 year, may be of value.

Second, the isotretinoin-associated changes in serum lipids and gamma glutamyl transferase (Lyons *et al.*, 1982) are reminiscent of changes caused by drugs which are known inducers of hepatic microsomal enzymes e.g., phenobarbitone (Miller & Nestal, 1973; Durrington *et al.*, 1976; Durrington, 1979), phenytoin (Pelkonen, Fogelholm & Nikkila, 1975; Nikkila *et al.*, 1978) and glutethimide (Bolton *et al.*, 1980). Interestingly, such enzyme-inducing drugs have been shown to decrease total thyroxine, free thyroxine and reverse tri-iodothyronine, but not tri-iodothyronine (Ohnhaus & Struder, 1983). The reduction in thyroxine with isotretinoin reported by Lyons *et al.* (1982) is further evidence that the drug may cause enzyme induction. This requires formal examination in view of the implications for the metabolism of other drugs.

Third, the link between serum lipids, enzyme induction and lipid turnover in the skin requires further exploration. It is curious that certain drugs which cause enzyme induction and a rise in serum lipids, e.g. phenytoin and phenobarbitone, are known to cause or exacerbate acne. Isotretinoin, which may fall into this category, occasionally causes eruptive xanthomas (Dicken & Connolly, 1980) despite its beneficial effects in acne by reducing skin sebum excretion rate (Lyons *et al.*, 1982).

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Scalp surface lipid

SIR, The letter by Drs Simpson and Martin (*British Journal of Dermatology*, 1983, **108**, 123) concerning our paper (Saint-Leger & Leveque, 1982) on scalp surface lipids suggests that the authors might have slightly misunderstood the objective of our work. The main aim of the study was not to confirm the homogeneity in the distribution of the lipids on the scalp, but to show that, for a given subject, the scalp sebum excretion rate (SER) is much lower than that obtained on the forehead.

We are fully aware of the difficulties in obtaining the follicular pattern on the scalp for obvious reasons which we ourselves outlined in our paper. Thus, in order to obtain an equivalent of the follicular pattern (i.e. a lipid-free scalp surface), we chose the easiest and most efficient procedure, that is, two successive standardized shampoos. Six hours later, the SER was then estimated on different zones of the scalp. It is worth noting that the preparation of the zones (by cutting the hair trim with the scalp surface) had been carried out, in some cases, just *before* shampooing.

As for a possible error arising during the sampling procedure this is unlikely, since in most cases, the successive samplings on the surface of the scalp followed the exponential law which has previously been described (Saint-Leger *et al.*, 1979). If there had been a failure in contact between the glass and the scalp, this law could not have been verified.

In conclusion, we do not believe that failure of contact could explain the large differences in SER between scalp and forehead which we observed, and we should have appreciated some mention of the SER values which Simpson and Martin obtained in their study.

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Book Reviews

Malignant Skin Tumours. Edited by J.J.Emmett and G.E.O'Rourke (1982) Edinburgh: Churchill Livingstone. Pp. 299. Price £35.00.

In the preface to this book, the editors, who are both surgeons, point out the need for wider dissemination of the information needed for the early diagnosis and adequate treatment of skin malignancies, which have greatly increased in frequency since 1940; they point out their mainly surgical approach in both the diagnostic and therapeutic sense. There are twenty-three contributors, including eight surgeons, six pathologists, one radiotherapist and six dermatologists.

Approximately half the book relates to standard tumour description, most of the rest being on the details of excision techniques. The title suggests a broad coverage of malignant skin tumours but only basal cell and squamous cell carcinoma and malignant melanoma are given detailed attention; the colour pictures of the clinical types are superb, but the same cannot be said of the histology sections.

It is always difficult to produce a book to cross speciality barriers; one feels that this volume has in fact fallen between too many stools. It will be most at home among surgeons, though one wishes that more attention had been given to simple techniques such as curettage and cryosurgery since there are many studies showing very good cure rates for appropriate tumour types.

R.P.R.Dawber

Clinics in Rheumatic Diseases: Cutaneous Lesions in the Connective Tissue Disorders. Vol. 8, No. 2. Edited by J.P.Callen (1982) London: W.B.Saunders Co., Ltd. Pp. 325–520. Price £10.75.

The advantage of recruiting specialists in particular fields to combine personal experience with a review of relevant up-to-date literature is well shown in this book.

The title might suggest that systemic lupus erythematosus and systemic sclerosis would be covered, but these disorders have formed the subject of previous volumes in this series of *Clinics in Rheumatic Diseases* (SLE, April, 1982; SS, April, 1979). The inclusion of urticaria, erythema multiforme, Sweet's syndrome and cutaneous reactions to rheumatological drugs is unexpected in a book on connective tissue diseases. There are also good chapters on nail capillary abnormalities and cutaneous immunofluorescence.

The two types of childhood dermatomyositis are well defined; the rare, severe and frequently fatal type with marked vasculitis, and the commoner inflammatory myopathy comparable to the adult form of this disease. The relationship of dermatomyositis and malignancy is fully discussed. The association occurs in about 24% of adult cases. Polymyositis alone is rarely associated with malignancy. Jeffrey Callen makes a good case for limiting investigations for malignancy in patients with dermatomyositis to clinical examination (including, in females, examination of the breasts and the pelvis), together with simple 'routine' tests such as erythrocyte sedimentation rates, blood count, radiology of the chest and examination of the faeces for occult blood. Unfortunately, he cannot answer the question 'When and how should the patient be re-evaluated for malignancy?' Patients should be re-investigated in the presence of new or unexplained symptoms, signs, laboratory abnormalities or exacerbations.

This is a practical book, worth buying for the departmental library.

N.R.Rowell

The Clinical Management of Melanoma. Edited by H.F.SEIGLER (1982) The Hague: Martinus Nijhoff. Number 5 in the *Series Developments in Oncology*. Pp. 522. Price $75.00.

In a book with this title one would expect to find reasoned and well-conducted discussion on current controversies concerning Surgical Management, Chemotherapeutic Approaches, Radiotherapy and possibly Immunotherapy in the management of patients with both primary malignant melanoma and more advanced disease. Examples of areas of controversy include the exact width of normal skin which requires to be excised from around a primary melanoma, and also the vexed question of prophylactic lymphadenectomy in patients with poor risk primary melanomas.

How well then does this text live up to such an expectation? The authors led by Dr Seigler, who is a surgeon with a special interest in immunology, all come from the Duke Medical Centre in North Carolina so this is in fact the Duke view of melanoma. Seventeen chapters cover very varying fields. Dr Seigler writes the first chapter on Surgical Considerations which is up to date and thoughtful. This is followed by a chapter on the Pathology of Melanoma which once again is reasonably up to date although the author introduces some concepts such as that of 'junctional melanoma' which are rather different from some of the current mainstream melanoma terminology. He does however make the very well worthwhile plea that room be left within the terminology for pathologists to state that they really do not know whether a lesion is an active naevus or an early melanoma. Following this there is an interesting chapter on Melanoma of the Genito-urinary Tract, a rarity, but comprehensively referenced. We then have 130 pages on Radiological Aspects of Thoracic Melanoma and also X-rays and barium studies illustrating the metastatic potential of this disease. Subsequent to this we have a chapter on Melanoma of the Eye and further on in the book there is a chapter on the Pathology of Melanoma of the Eye. More brisk editorial control and the bringing together of these two chapters would have made their reading easier and more logical. Similarly the two chapters on Immunobiology and Immunotherapy of Melanoma should have been put together and some natural flow between the two allowed to occur. The only colour pictures in the book are found in the chapter on Dermatological Manifestations of Melanoma and these are of high quality as is the rest of the chapter.

For myself however the highlight of the book was a very reasoned and interesting chapter on Hormonal Aspects of Melanoma by McCarty and colleagues. This discusses in an enlightened and up-to-date manner the possible role of hormonal influences in both the induction and progression of malignant melanoma and is I think one of the best available accounts of hormonal influences in melanoma.

The book is sturdily bound and the illustrations are of high quality.

With finance for hospital and university libraries in its present sorry state would I recommend purchase of the book for such institutions? This is difficult to answer and will very much depend on the interest of individuals working in such groups. I would suggest that anyone who has an active interest in melanoma should look at the book and see if any of the individual chapters merit its purchase.

RONA M.MACKIE

Infectious Diseases Illustrated. By H.P.LAMBERT and W.E.FARRAR (1982) Oxford: Pergamon Medical Publications. Price £48.00.

The diagnosis of most infectious diseases depends on a critical appraisal of symptoms and physical signs, X-rays and simple laboratory procedures such as smears and cultures. In this book colour photographs illustrating the most important clinical and laboratory features of infectious diseases are brought together with a short text to provide a comprehensive guide to the subject. The book is divided into sections dealing with infections of specific organ systems. Cutaneous features of infections are amply covered. In addition there are representative illustrations of gross pathology and histopathology. There are separate sections on parasitic infections and sexually transmitted diseases. The last chapter is a brief illustrated introduction to laboratory studies such as the electronmicroscopic appearances of different virus particles and gas chromatographic analyses of bacterial antigens.

The quality of the colour illustrations in *Infectious Diseases Illustrated* is generally excellent. The reproduction of the X-rays, which is so often a problem in books, is clear. There are a large number of photographs of the skin manifestations of both localized and systemic infections ranging from erysipelas to staphylococcal septicaemia. Infections of the eye are also covered. Photographs of different diseases are accompanied by a short description of the clinical and laboratory features of the infection as well as its management.

This book will be of great value to the dermatologist. It will find a particular use in the recognition of cutaneous manifestations of systemic infections. All the important infections are covered and many of the rarer diseases are also illustrated. In this latter group are a number of conditions such as Kawasaki disease and cat scratch fever which are seldom illustrated.

The text is not intended to be a substitute for more detailed accounts of infectious diseases. However it is a convenient source of information on the essential clinical and laboratory features of these conditions. The price of the book is £48.00—a figure which presumably reflects the large number of coloured plates. A slide atlas based on the material in the book is also available.

R.J.HAY

Unwanted Effects of Cosmetics and Drugs used in Dermatology. By JOHAN P.NATER and ANTON C. DE GROOT (1983) Amsterdam: Excerpta Medica. Pp. 483. Price $106.50.

In the investigation of dermatological patients it is essential to consider whether a cutaneous reaction could be due to a drug, a cosmetic or other external cause. This book is a mine of information on the many and various side-effects of cosmetics and drugs used in dermatology. The format of the book is brief descriptions and many tables giving comprehensive references for every allergen, irritant and side-effect described. Patch testing concentrations and vehicles are included in the tabulations.

The first chapters discuss the technique and interpretation of patch testing, although the final 3-day reading recommended would not be considered by everyone to be conclusive.

Then follows a comprehensive compilation of all the sensitizers identified in the bases and active ingredients of topical medicaments. Topical phototoxins and photoallergens are listed and the causes of unusual forms of contact dermatitis including purpuric, pigmented and follicular dermatitis, erythema multiforme-like eruptions, granulomas and toxic epidermal necrolysis.

Subsequent chapters give the causes of contact urticaria and drugs causing necrosis, acne and discoloration. Then follows a succinct but comprehensive review of the side-effects of thirty-five topical medicaments, beginning with arsenic, including boric acid, clindamycin, dithranol and hexachlorophene and finishing with the triphenylmethane dyes. The medicament chapters conclude with the side-effects of many systemic drugs.

The second half of the book deals with cosmetics and includes incidences and causes of reactions and methods of patch testing. Every type of cosmetic is considered in detail, giving sample formulations, frequency of adverse reactions, lists of contact allergens and other types of side-effect. One chapter lists the plant products used in cosmetics and the side-effects reported. The final chapter of more than a hundred pages lists the vast array of cosmetic ingredients, their synonyms, patch test concentrations and vehicle, frequency of use, reports of contact allergy and appropriate cross-references.

To gather so much information into such a tidy volume is a great achievement. The information is clearly set out, the compounds are listed alphabetically, the references are up to date and the cross referencing is simple and I gratefully say never irritating. This is an excellent book and I strongly recommend it to all dermatologists and also to the pharmaceutical and cosmetic industries. We all certainly need it.

E.CRONIN

British Journal of Dermatology (1983) **109,** 129–130.

News and Notices

Gordon Research Conference on the Epidermis, New Hampshire, August 1983

A Gordon Research Conference will be held at Tilton, New Hampshire on the subject of epithelial differentiation and keratinization on 8–12 August 1983. Dr W.L.Epstein will be the Chairman and Dr H.Baden will be the Vice-Chairman. Requests for applications to the Conference or for additional information should be addressed to: Dr A.M.Cruickshank, Director, Gordon Research Conferences, University of Rhode Island, Kingston, Rhode Island 02881, U.S.A.

Biological Sciences relating to Dermatology, Cambridge, September 1983

A course on 'Progress in the biological sciences in relation to dermatology' will be held at Churchill College, Cambridge from 26 to 30 September 1983. It is 25 years since the first of these courses was held in Cambridge. They have been held every 5 years since 1958 and the programme differs each time. They are primarily aimed at bringing consultants in clinical dermatology up to date with relevant advances in the basic sciences but dermatologists in training and others interested in the skin are welcome to attend.

Closing date for applications: 26 August, 1983. Further details may be obtained from: The Conference Secretary, Postgraduate Medical Centre, Clinical School, Addenbrooke's Hospital, Cambridge CB2 2QQ.

2nd European Symposium on Psoriasis, Trieste, October 1983

The 2nd European Symposium on Psoriasis, co-sponsored by the European Society for Dermatological Research and the Italian Society of Dermatology and Venereology, will be held at the Adriatico Palace Hotel, Trieste, Italy, on 7–9 October, 1983. The fee for registration (if received before 31 July, 1983) is 150,000 Liras. Further information from Dr F.Kokelj, Clinica Dermatologica Universitaria, c/o Ospedale Maggiore, Via Stuparich, 1 34125 Trieste.

Investigative Techniques in Dermatology Course, Cardiff, November 1983

The 5th Investigative Techniques in Dermatology Course will be held at the Welsh National School of Medicine, Cardiff on 16, 17 and 18 November 1983. The course is designed to give insight into some of the basic research techniques applicable to dermatology, and also into modern methods for the investigation of patients with skin disease. For further details please apply to: Professor R.Marks, Professor of Dermatology, Dept. of Medicine, Welsh National School of Medicine, Heath Park, Cardiff CF4 4XN.

Biology of the Skin, Cambridge, December 1983

The annual 'Introductory course on the biology of the skin' will be held in Fitzwilliam College, Cambridge, from 5 to 9 December 1983. This course is intended primarily for registrars and postgraduate students at an early stage of their training in dermatology, but is open to other medical practitioners who are interested.

Closing date for applications: 7 November 1983. Further information may be obtained from: The Conference Secretary, Postgraduate Medical Centre, Clinical School, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ. Telephone: Cambridge (0223) 212216.

ESDR Symposium on 'The Urticarias', Cambridge, 1984

A 2-day ESDR Symposium on 'The Urticarias' will be held in Robinson College, Cambridge, on 10 and 11 January 1984. The basic mechanisms of urticaria and clinical aspects will be discussed. Details relating to the scientific programme may be obtained from Professor M.W.Greaves, Institute of Dermatology, Homerton Grove, London E9 6BX. Abstracts of about 200 words for Free Communications and Posters should be sent to Professor M.W.Greaves before 1 October 1983.

Information regarding registration etc., may be obtained from Mrs H.Hatfield, Postgraduate Medical Centre, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ, England. Tel. Cambridge (0223) 212216. Closing date for registration: 1 November 1983.

Models to Study Function and Disease of the Skin, Cardiff, March 1984

An International Symposium (sponsored by the European Society for Dermatological Research) entitled 'Skin Models–Models to Study Function and Disease of the Skin' will be held at Cardiff on 15, 16 and 17 March 1984. The symposium organizers are: Prof. R.Marks of the Welsh National School of Medicine, Prof. G.Plewig of the University of Düsseldorf and Prof. H.Maibach of the University of San Francisco. The meeting will include invited lectures, free communications, poster papers and workshops. The symposium should be useful for dermatologists, pharmacologists, toxicologists, cosmetic scientists and others interested in skin biology. For further details, abstract and registration forms, please contact Professor R.Marks, Professor of Dermatology, Dept. of Medicine, Welsh National School of Medicine, Heath Park, Cardiff, CF4 4XN.

Dermatology Conference, Singapore, May 1984

The Sixth Regional Conference of Dermatology (Asian–Australasian) will be held in Singapore on 5–9 May 1984. For further information please contact the Secretariat, Sixth Regional Conference of Dermatology, Academy of Medicine, 4a College Road, Singapore 0316, Republic of Singapore.

Denis Thienpont Prize in Parasitology and Mycology

The Denis Thienpont Prize, amounting to U.S. $25,000 will be awarded in 1985, under the auspices of the 'Koninklijke Academie voor Geneeskunde van Belgie' and the 'Académie Royale de Médecine de Belgique'. The prize will be awarded to an investigator presenting original work concerned with fundamental or clinical research in parasitology or mycology. Applications must be made on the appropriate form which may be obtained from the Secretary's office of the 'Koninklijke Academie voor Geneeskunde van Belgie', Hertogsstraat I, 1000 Brussels. Six copies of the submitted work should be enclosed before 31 December 1984.

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NOTICE TO CONTRIBUTORS

The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes signed editorials reviewing, primarily for the benefit of the clinician, advances in laboratory research, and also reviews of recent advances in such aspects of dermatology as contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews, correspondence and society proceedings. The Journal is the official organ of the British Association of Dermatologists and also of the Netherlands Association for Dermatology and Venereology, but it attracts contributions from all countries in which sound research is carried out, and its circulation is equally international.

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Manuscripts should be sent to the Editor, Dr J.L.Burton, Dermatology Department, Bristol Royal Infirmary, Bristol BS2 8HW. They should be typewritten on one side of the paper only, with a wide margin, be double spaced, and bear the title of the paper, name and address of each author, together with the name of the hospital laboratory or institution where the work has been carried out. The name and full postal address of the author who will be responsible for reading the proofs should be given on the first page. A duplicate copy, complete with figures, tables and references, should be sent, since in many cases two referees are consulted. The author should keep a carbon copy of his manuscript. The Editorial Board reserves the right to make literary corrections.

Abbreviations and units. Full names with uncommon abbreviations must be given with the first mention: new abbreviations should be coined only for unwieldy names and should not be used at all unless the names occur frequently. In the title unusual abbreviations should be identified, in the introduction and discussion they should be used sparingly. Abbreviations of units should conform with those shown below.

gram(s)	g	minute(s)	min	molar	mol/l
kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3}g)	mg	second(s)	s	gravitational acceleration	***g***
microgram(s) (10^{-6}g)	μg	cubic millimetre(s)	mm^3	micrometre(s)	μm
nanogram(s) (10^{-9}g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12}g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^{131}I
hours(s)	h	milliequivalent	mmol		

These and other abbreviations and symbols should follow the recommendations of: *Units, Symbols and Abbreviations, A Guide for Biological and Medical Editors and Authors* (1977) The Royal Society of Medicine, London.

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PL 0173/0030 PA 278/7/1

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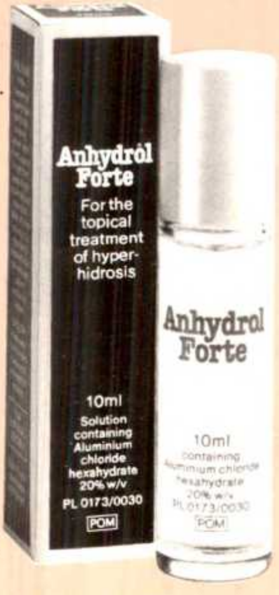
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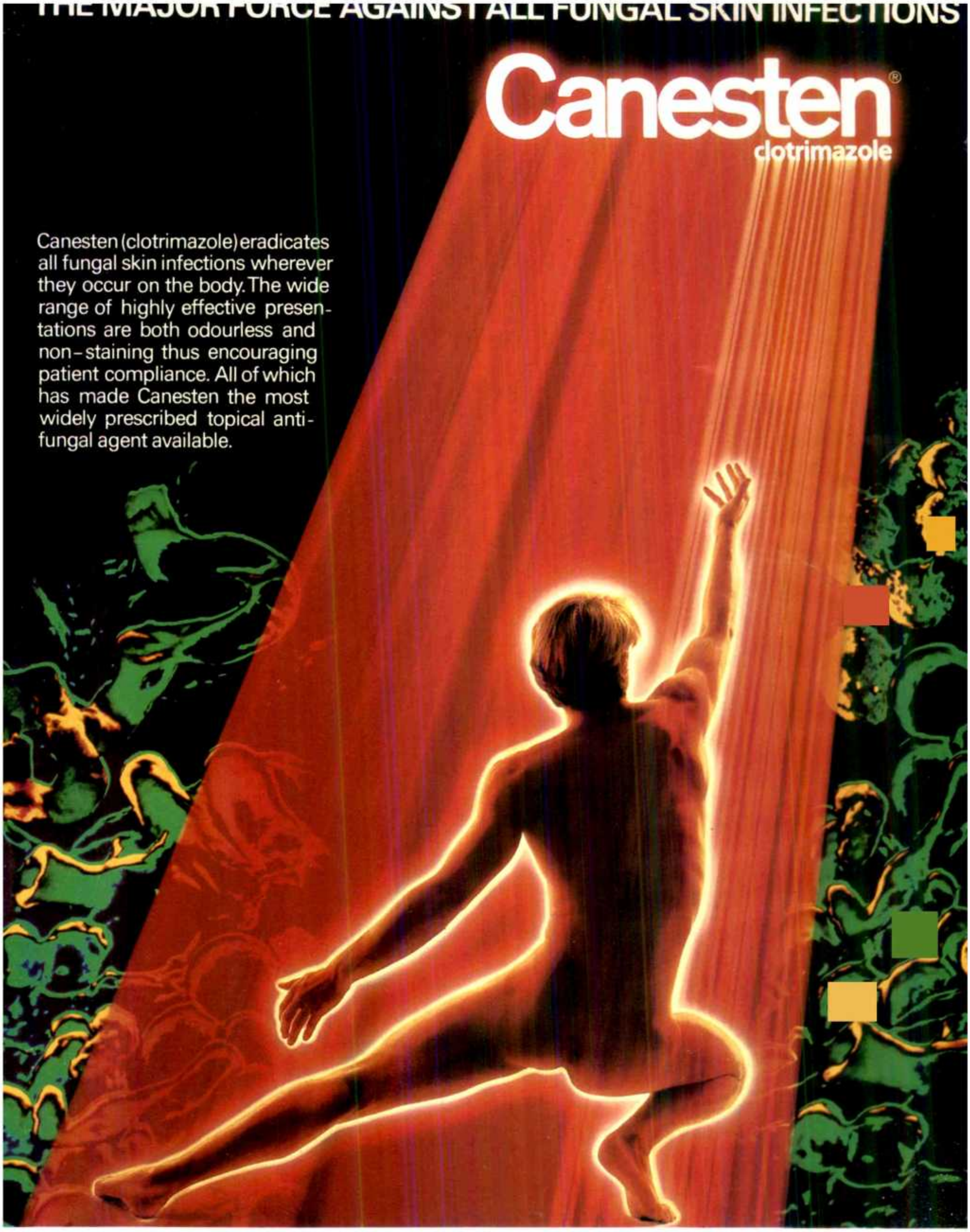

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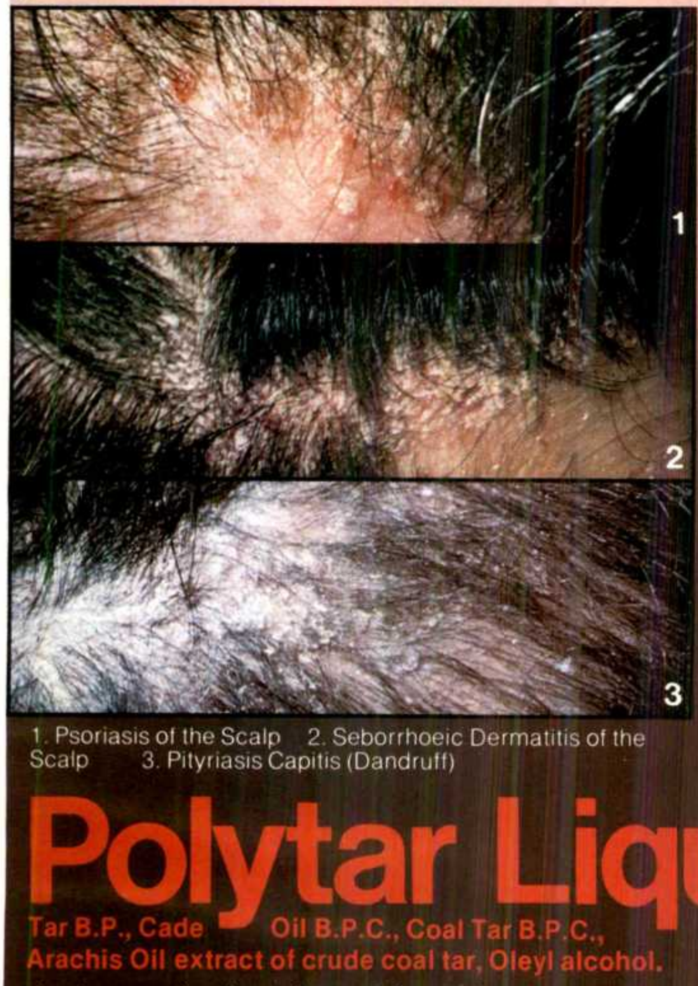

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Polytar Plus contains 3.0% hydrolysed animal protein, in addition to the above.

Uses Polytar Liquid and Polytar Plus are indicated in the treatment of scalp disorders such as psoriasis, seborrhoea, dandruff, eczema and pruritus. They are also of value in the removal of pastes and pomades used in the treatment of psoriasis.

Dosage and Administration The hair should be wetted and sufficient applied to produce an abundant lather. The scalp and adjacent areas should be vigorously massaged with the fingertips. The hair should then be thoroughly rinsed and the procedure repeated.
Polytar Liquid or Polytar Plus should be used once or twice weekly.

Contra-indications, warnings, etc. Nil

Pharmaceutical Precautions Store in a cool place

Packs and Prices

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Product Licence Number
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Reference: 1. Medicine 1977; 32: 1857.

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Fry L. Br Med J 1982; 284:1093—109

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effects

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vided the weekly dosage of Dermovate is less than 50 ns in adults, any pituitary-adrenal suppression is likely to be ent with a rapid return to normal values once the short f steroid therapy has ceased. The same applies to given proportionate dosage.

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Presentation	**Product licence number**
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etnovate Scalp Application
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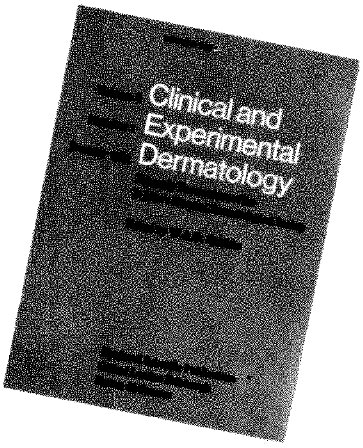

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1. Mims Magazine, 15th May 1982

Prescribing Guidelines
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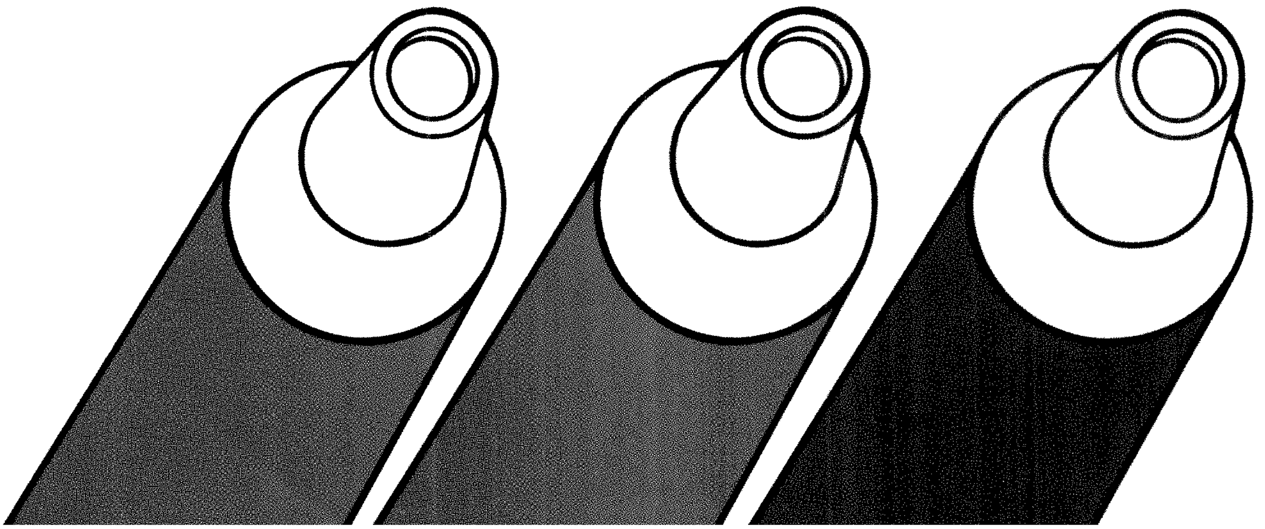

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Clinical and Laboratory Investigations

Clues to the aetiology and pathogenesis of herpes gestationis

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SUMMARY

In a study of twenty-five patients with herpes gestationis we found that 80% possessed the HLA antigen DR3, which confers increased immune responsiveness and a predisposition to 'auto-immune disease'. In five patients the development of herpes gestationis coincided with a change in sexual partner, suggesting that the development of herpes gestationis may depend on exposure to an antigen derived from the father. This might share determinants with a component of the basement membrane zone of skin. Although anti-basement membrane zone antibodies are present in HG it is not clear whether they play a pathogenic role. The infrequency of neonatal involvement and the lack of correlation between immunofluorescence findings and clinical activity in our patients suggested that the antibodies might be a result of tissue damage rather than its cause. Two patients in our study were exceptional in that episodes of herpes gestationis were followed by normal pregnancies. In these patients the relationship of their DR antigens to those of the fetus may have been important in determining whether or not the pregnancy would be affected by herpes gestationis.

The aetiology of herpes gestationis (HG) is unknown, but recent investigations have highlighted the importance of immunopathological features in this disease. We have now examined the clinical and immunological findings in twenty-five patients affected by HG in forty-four pregnancies in an attempt to clarify its aetiology and pathogenesis.

METHODS

The twenty-five patients with HG were submitted to our national survey by dermatologists throughout the United Kingdom. All the patients had a history of an intensely pruritic urticarial

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and bullous eruption in association with pregnancy (Holmes *et al.*, 1982). The diagnosis of HG was supported by positive direct or indirect immunofluorescence (IMF) for C3 at the basement membrane zone (BMZ) in all cases except one (K.W.) who had HG prior to the introduction of IMF techniques.

All the twenty-five patients were seen by one of us (R.C.H.). A detailed history was taken to determine the effect of parturition, breast feeding, menstruation, and the oral contraceptive on the clinical activity of HG. The frequency of neonatal involvement was noted. The patients were assessed clinically to determine whether they had any associated auto-immune disorders. Blood was taken for indirect immunofluorescence (IMF), auto-antibodies, HLA antigens, and HLA antibodies. HLA typing was performed using the standard NIH two-stage, complement-dependent, microcytotoxicity test (Kissmeyer-Nielsen & Kjgrbye, 1967); the standard B lymphocyte technique (Bodmer *et al.*, 1978) was used for DR typing. The A and B antigens were compared with the findings in 300 normal controls and the DR antigens with 221 normal controls. The results were analysed using the χ^2 test. As fifty different antigen comparisons were performed, only P values of less than 0·001 were considered significant. All quoted P values are two-tailed. The frequency of HLA antibodies, auto-antibodies and auto-immune disorders was compared with a control group of thirty-three women with normal pregnancies. In twelve patients HLA typing was performed on their sexual partners and in three patients the children were also studied. Skin biopsies were taken from normal appearing skin for direct IMF during the active stage of the eruption and also at variable intervals after the cessation of clinical activity. IMF studies were performed using standard direct and indirect techniques (Beutner & Nisengard, 1973; Carruthers, Black & Ramnarain, 1977).

RESULTS

In ten patients HG developed with the first full term pregnancy. Among the fifteen patients where it began in the second or subsequent pregnancy there were five patients in which the onset

TABLE 1. HG: The possible aetiological importance of the sexual consort

	Case				
Pregnancy	P.T.	P.S.	G.F.	P.G.	C.A.
1	A−	C−	E−	G−	J−
2	B+	C−	E−	H−	J−
3		D+	E−	H−	J−
4			F+	H−	J−
5				I+	J−
6					J−
7					J−
8					K−
9					L+

A, B, C, etc.: Sexual consorts;
+: Development of HG;
−: No development of HG.

TABLE 2. HG: Summary of the HLA findings

Antigen	Frequency	Probability
A1	76%*	0·0003
B8	76%*	0·00002
DR3	85%†	0·00005
DR4	50%†	0·10
DR3 + DR4	45%†	0·00005

*n = 25;
†n = 20.

coincided with a change in sexual partner (Table 1). In one case (J.E.) HG developed in her first pregnancy and it recurred in two subsequent pregnancies by a second husband.

Clinical exacerbations occurred immediately after delivery in 55% (22/40) of pregnancies while in four pregnancies HG began post-partum. In the thirteen episodes of HG where the patients breast fed, the average duration post-partum was 5 weeks for the bullous lesions and 24 weeks for the urticarial rash. This compared with 35 weeks for the bullous lesions and 68 weeks for the urticarial rash in the patients who exclusively bottle fed their infants. In sixteen patients the clinical activity of HG varied with menstruation; the exacerbations were pre-menstrual in twelve and mid-cycle in four. The oral contraceptive was prescribed to eight patients and in four this produced a clinical relapse. Three of the patients who had relapsed on the pill had been prescribed a combined oestrogen and progestogen preparation. The fourth patient, who had a

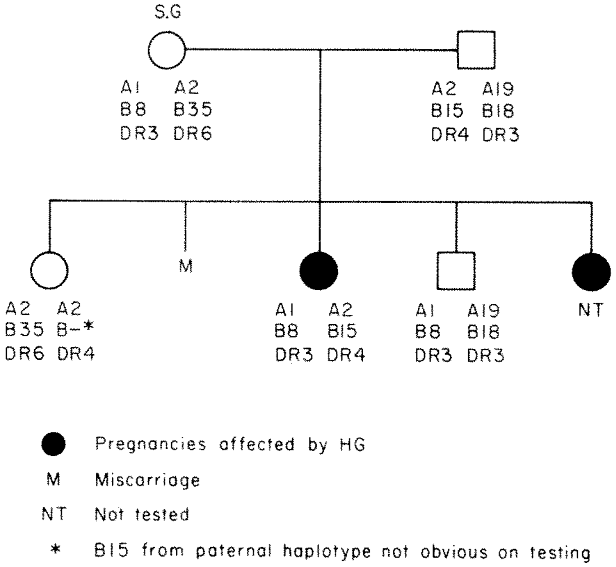

FIGURE 1. Case S.G. HLA family study.

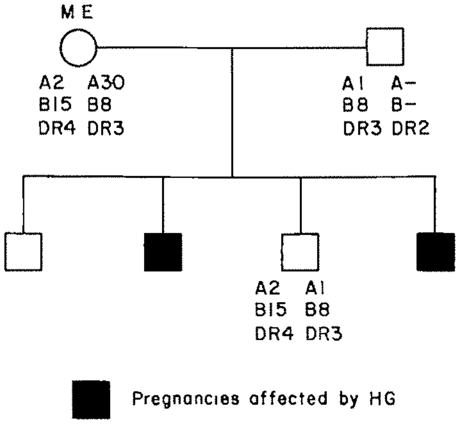

FIGURE 2. Case M.E. HLA family study.

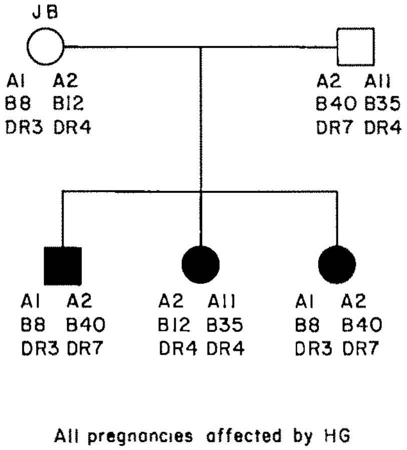

FIGURE 3. Case J.B. HLA family study.

particularly severe flare of her HG, had been prescribed oestrogen alone. Two of our patients were prescribed a progestogen alone and this had no effect on their HG.

The frequency with which the neonates were affected by the bullous eruption was 2% (1/44).

The significant HLA findings in HG are summarized in Table 2. In the sexual consorts of patients with HG there were no significant HLA associations. In two patients (S.G. & M.E.) episodes of HG were followed by completely unaffected pregnancies with recurrence of HG in subsequent pregnancies. HLA findings in their husbands and children are shown in Figs 1 and 2. The HLA findings in the family of case J.B. who had three affected pregnancies are shown in Fig. 3. Twenty-one patients had their serum examined for HLA antibodies. These were present in one primigravida patient and in 50% (10/20) of the multigravida patients. HLA antibodies were found in 6% (1/16) of primigravida and 22% (4/18) of multigravida controls during pregnancy.

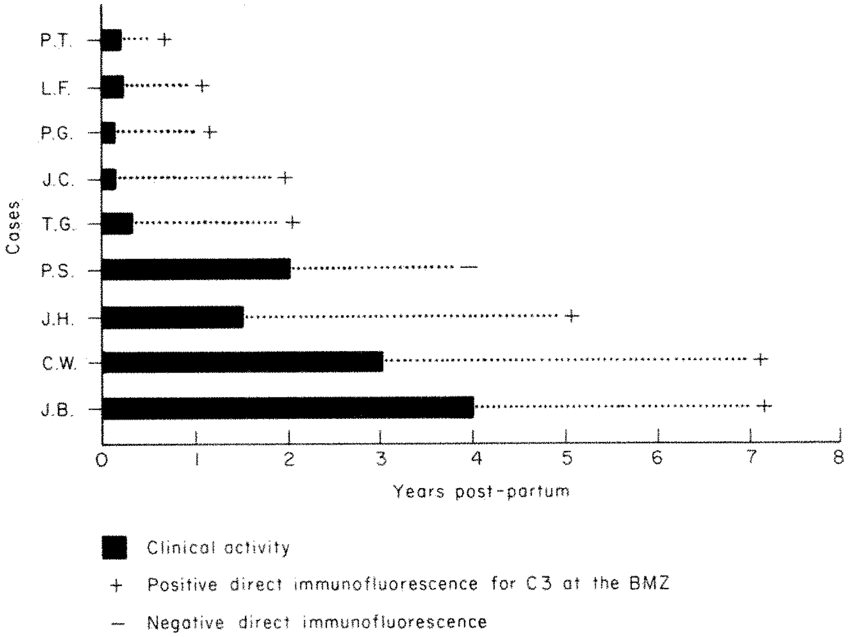

FIGURE 4. Direct immunofluorescence findings in normal appearing skin after the clinical resolution of herpes gestationis.

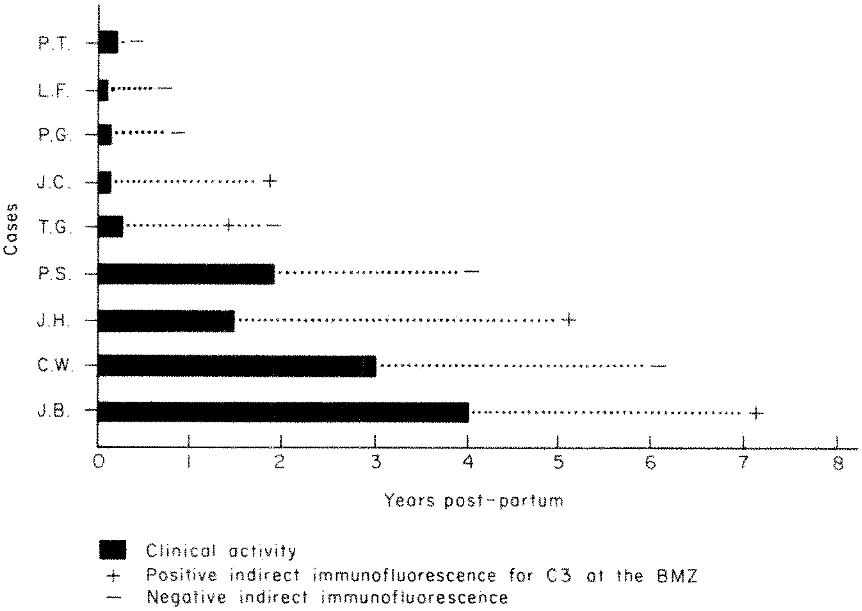

FIGURE 5. Indirect immunofluorescence findings after the clinical resolution of herpes gestationis.

Deposition of C3 at the BMZ was demonstrated in 100% (23/23) of patients by d and 80% (16/20) by the indirect technique. IMF for IgG at the BMZ was positive in 2 by direct and 25% (5/20) by indirect IMF. The severity of HG did not correlate with 'HG factor' titre or positive IMF for IgG. The duration of positive direct IMF for BMZ is shown in Fig. 4 and indirect IMF in Fig. 5.

In our patients with HG, five developed Graves' disease ($P=0{\cdot}025$) two alope ($P=0{\cdot}34$) and one vitiligo ($P=0{\cdot}88$); in thirty-four control subjects there was nc of associated auto-immune disorders. There was an increased frequency of thy antibodies in our patients compared with pregnant controls (thyroid microsomal, thyroglobulin, $P=0{\cdot}004$).

DISCUSSION

Our patients with HG demonstrated a significant increase in the frequency of the HL A1, B8 and DR3 (Table 2). An association between HG and DR3 has also been re Shornick, Stastny & Gilliam (1981). The antigens A1, B8 and DR3 demonstra disequilibrium and of this haplotype it is the association with DR3 which is probabl important aetiologically. The explanation of disease associations with DR3 remains u increase in the frequency of DR3 has been found in several diseases which appear immunological pathogenesis including dermatitis herpetiformis, coeliac diasese, syndrome, Addison's disease, Graves' disease and myasthenia gravis (Svejgaard *et al* is known that the DR antigens control cooperation between macrophages, T-helpe cytes and B lymphocytes and this suggests that they may be identical to the immun (Ir) determinants, or alternatively they may be in close linkage disequilibrium (McDevitt & Bodmer, 1972; McDevitt & Bodmer, 1974). Therefore Batchelor (1980 that the disease associations with DR3 could depend on an HLA DR3-related Ir broad specificity which conferred a general heightening of immune responsiveness. of this he cited several diseases in which patients with B8 or DR3 had increase responses compared with patients without B8 or DR3. Also diseases such as alcoholi rheumatoid arthritis and Graves' disease appear to run a more severe course in patien or DR3 (Bailey *et al.*, 1976; McGregor *et al.*, 1980). In our patients with HG, in additi increased frequency of DR3, there was also a significant increase in the frequency v DR3 occurred in combination with DR4 (Table 2). Shornick *et al.* (1981) have also association. As DR4 is associated with disorders such as insulin-dependent rheumatoid arthritis and hydrallazine-induced SLE (Svejgaard *et al.*, 1981), it might to DR3 in conferring a susceptibility to immunological disease, making individuals DR3 and DR4 particularly at risk. Therefore HG appears to primarily affect wom immunogenetically susceptible to immunological disease. This would accoun observation that HG was associated with Graves' disease and an increased frequency auto-antibodies.

In our study the development of HG was always related to pregnancy; however, been described in association with hydatidiform mole (Dupont, 1974) and chorio (Tillman, 1950). A feature which is common to pregnancy and the trophoblastic tum the patient is exposed to foreign antigens derived from her sexual consort. This sugge HG the sexual partners might be important aetiologically. Considerable support f provided by five of our patients in which the development of HG coincided with a sexual partner (Table 1). Investigation of the sexual consorts to determine wheth share a common antigenic factor which could trigger HG has so far been limited to HLA studies. Shornick, Stastny & Gilliam (1982) found an increased frequency of HLA DR2 and several studies have drawn attention to the fact that women with a tendency to HG have HLA antibodies directed against their husbands' HLA antigens (Reunala *et al.*, 1977; Eberst *et al.*, 1981; Shornick *et al.*, 1982). However, in our patients the frequency of HLA antigens in the sexual consorts was normal and although the women with HG had an increased tendency to produce HLA antibodies we felt that this was probably the result of their high immune responsiveness rather than the cause of the HG. The antigenic trigger for HG remains unidentified, but we presume that it shares antigenic determinants with a component of the BMZ of skin and, in this way, induces an immunological response which cross-reacts with the skin. As HG is associated with trophoblastic tumours, as well as normal pregnancy, it is possible that the antigenic trigger for HG resides on trophoblastic cells or cells of the chorio-amniotic membranes. If HG is the result of an immunological reaction directed primarily against chorio-amniotic cells, but cross-reacting with skin, this may explain why HG so often begins at the umbilicus. In fetal life the skin at the umbilicus is in continuity with the chorio-amniotic membrane covering the umbilical cord and therefore in adulthood there may be cells in the region of the umbilicus which retain some of their chorio-amniotic antigenicity.

Once HG has developed it usually recurs in all further pregnancies. Two of our patients were unusual (cases S.G. and M.E.) in that HG failed to develop in a subsequent pregnancy. Our HLA studies confirmed that, in each of these two cases, the husband was the father of both the affected and unaffected pregnancies (Figs 1 and 2). One would have expected that the hormonal changes of pregnancy would have been sufficient to reactivate their HG. The fact that HG failed to occur in patients who had previously been affected suggested that HG was actively suppressed in the 'skipped' pregnancies. As HG is thought to be immunologically mediated its failure to develop could have resulted from the activation of suppressor T cells. The activation of these cells is probably associated with immune determinants which are closely linked to the DR antigens. Therefore it may be relevant that in both of the pregnancies which were spared by HG the fetus had DR antigens which were also possessed by the mother (Figs 1 and 2). This could have favoured the induction of a suppressor T-cell response. Usually the antigenic trigger for HG is in the company of a foreign DR antigen and this might enhance the auto-immune process. However, in an HLA study of a further family in which all of the pregnancies were affected by HG (J.B.) we demonstrated that HG could occur when the DR antigens of the fetus were shared by the mother (Fig. 3, second pregnancy). Thus the explanation as to why HG 'skipped' pregnancies in cases S.G. and M.E. remains uncertain.

The activity of HG is undoubtedly hormonally modulated. This is illustrated by the exacerbations which may occur with the oral contraceptive (Lynch & Albrecht, 1966; Mitchell, 1966). In our study eight patients were prescribed an oral contraceptive and four experienced exacerbations. Three of the patients with exacerbations had been given a combined oestrogen and progestogen pill, but the patient who flared most severely of all had been given oestrogen alone. In the two patients who were given a progestogen alone their HG was unaffected. This would indicate that it is the oestrogen component of the oral contraceptive which is primarily responsible for exacerbations of HG. There is experimental evidence that at certain concentrations oestrogen may have immuno-enhancing properties (Kenny, Pangburn & Trail, 1976) and this may account for its effect in HG.

Changes in severity of HG with menstruation were demonstrated in sixteen of our patients. In four the exacerbations occurred with ovulation, which is in accord with our suggestion that oestrogen is responsible. However, in twelve patients the exacerbations occurred premenstrually when the most significant hormonal change is a fall in progesterone. As progesterone has been shown to have immunosuppressive effects similar to glucocorticoids (Siiteri *et al.*, 1977; Clemens, Siiteri & Stites, 1979) it should exert an inhibiting effect on HG. Therefore the fall in progesterone which occurs premenstrually might be the factor which allows HG to flare at this time. The effects of progesterone could also account for the changes in severity of HG which occur during pregnancy. HG is usually in a phase of relative remission in the last few weeks of pregnancy when the progesterone level is high, but when the progesterone level falls abruptly immediately post-partum there is often a severe flare in HG (Holmes *et al.*, 1982). Changes in disease activity following parturition are seen in other disorders with an immunological pathogenesis including systemic lupus erythematosus (SLE) (Garsenstein, Pollak & Kark, 1962) and leprosy (Rose & McDougall, 1975). SLE frequently flares post-partum and in leprosy the type 1 lepra reaction is more common. Both findings could be related to the fall in progesterone after parturition and consequent increase in cell-mediated immunity.

It has been suggested by Bazex *et al.* (1981) that prolactin may exacerbate HG. They reported a patient who was treated with bromocriptine following delivery with apparent clinical benefit and they also considered that HG was more severe in their patients who breast fed. However, in our study prolactin did not appear to exacerbate HG as the women who breast fed tended to be less severely affected.

Our IMF studies demonstrated that all patients had C3 deposited at the BMZ in normal appearing skin and a circulating HG factor was present in 80%. If this HG factor were of pathogenic importance one would expect clinical remission post-partum to coincide with disappearance of the anti-BMZ antibodies, but this was not the case in our patients. In nine patients where direct IMF for C3 was performed on normal skin at intervals ranging from 6 months to 4 years after the cessation of clinical activity, IMF had remained positive in eight patients (Fig. 4). Similarly indirect IMF remained positive long after the cessation of clinical activity in a number of patients (Fig. 5). In addition, the HG factor titre bore no relationship to clinical activity. Therefore our IMF studies merely confirmed the presence of the HG factor and did little to confirm a pathogenic role for this antibody. A feature of HG which has been used to support the pathogenicity of the HG factor is the occurrence of neonatal HG with positive direct IMF (Chorzelski *et al.*, 1976; Katz *et al.*, 1977). However, as the HG factor is an IgG (Jordon *et al.*, 1976) we know that it will cross the placenta in all cases. If it were pathogenic then neonatal involvement should be common, whereas in our study only one child out of forty-four was affected. This suggested to us that neonatal HG might be mediated by cytotoxic T lymphocytes as these would cross the placenta with less consistency than antibody. In view of these various findings the role of the HG factor in the pathogenesis of HG must remain in doubt.

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Immunofluorescence identification of eosinophil granule major basic protein in the flame figures of Wells' syndrome

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SUMMARY

An indirect immunofluorescence assay using formalin-fixed paraffin-embedded skin was performed on six biopsies from four patients with eosinophilic cellulitis (Wells' syndrome) to determine the extracellular localization of eosinophil granule major basic protein (MBP). Serial sections from each biopsy were treated with either affinity chromatography-purified antihuman-MBP or staphylococcal protein A purified rabbit IgG (control material). There was striking extracellular fluorescence localized to flame figures, and intracellular staining of eosinophils in all sections treated with anti-MBP as compared with controls. The pattern of MBP extracellular staining corresponded to the configuration of each flame figure (as verified by counterstain of the same section with haematoxylin and eosin). These findings show that MBP can be used as a marker for determining eosinophil degranulation and, because MBP is localized to flame figures, they suggest that MBP may play a pathogenic role in Wells' syndrome.

Wells (1971) described a group of patients with recurrent oedematous and infiltrative plaques manifesting a characteristic histological picture including diffuse infiltration with eosinophils, histiocytes, and foci of amorphous and/or granular eosinophilic material associated with connective tissue fibres of the dermis, so-called 'flame figures'. The aetiology of this syndrome is unknown, but the histology suggests a pathogenic role for the eosinophil.

The eosinophil granule contains an electron-dense core and electron-lucent matrix. Human major basic protein (MBP), molecular weight 9200, constitutes the core and accounts for 50% of the granule and 25% of the total cellular protein (Gleich *et al.*, 1976; Lewis *et al.*, 1978; Gleich *et al.*, 1980; Ackerman, Durack & Gleich, 1982). Eosinophils are capable of damaging parasites, in particular *Schistosoma mansoni* and *Trichinella spiralis*, through release of granule contents on to the organism's surface, and injury to the parasite is mediated by MBP

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(Butterworth *et al.*, 1979; Wassom & Gleich, 1979). Additional evidence for the cytotoxic nature of MBP is provided by studies showing the ability of MBP to directly lyse a number of mammalian cells (Butterworth *et al.*, 1979; Gleich *et al.*, 1979). MBP also has been shown to mimic the changes seen in bronchial asthma, including dose-related decreased ciliary beating and exfoliation of mucosal cells (Frigas, Loegering & Gleich, 1980; Frigas *et al.*, 1981; Filley *et al.*, 1982).

We performed an indirect immunofluorescence assay utilizing affinity chromatography-purified antihuman-MBP antibody applied to formalin-fixed, paraffin-embedded skin biopsy sections from four patients with Wells' syndrome to determine whether eosinophil degranulation, as judged by the presence of MBP external to the eosinophil, is associated with the histopathological features of eosinophilic cellulitis. The results support a pathogenic role for eosinophils and for the cytotoxic MBP in Wells' syndrome.

METHODS

Six biopsies from four patients were selected from the dermatopathology files on the basis of histological criteria described by Wells, including dermal eosinophilia, flame figures, and histiocytes palisading around the areas of apparently altered collagen (Wells, 1971; Wells & Smith, 1979). The clinical history and histology of patients 3 and 4 have been previously reported from our department (Spigel & Winkelmann, 1979).

The indirect immunofluorescence assay, utilizing formalin-fixed, paraffin-embedded biopsies, has been described previously (Filley, Ackerman & Gleich, 1981; Filley *et al.*, 1982). We have modified this procedure by using affinity chromatography-purified reagents (Peters *et al.*, 1983). Six-micron serial sections were obtained from each paraffin block and affixed to glass slides with LePage's Bond Fast resin glue (LePage's Ltd., Bramalea, Ontario, Canada). After deparaffinization in xylene and rehydration through alcohol and water, the tissues underwent trypsin digestion at 37°C for 1 hour (Brozman, 1978; Huang, Minassian & More, 1976). The slides were then washed in water and placed in 10% normal goat serum (Pel-Freez Biologicals, Rogers, AR) for overnight incubation at 4°C. On day 2, slides were washed in Dulbecco's phosphate-buffered saline (PBS), the area surrounding the tissue was dried, and the biopsy sections were circled utilizing a Blaisdell yellow china marker (170T, Berol Products Division, Berol Corp., Danbury, CT). Two serial sections from each biopsy were prepared in the above manner; one was then overlaid with 250 microlitres of affinity chromatography-purified rabbit antihuman MBP, and the other overlaid with 250 microlitres staphylococcal protein A purified rabbit IgG as control material (Goding, 1978). The slides were placed in an incubation chamber overnight at 4°C. On day 3, after washing in PBS, slides were incubated for 30 min in 1% chromotrope 2R at room temperature. After a second PBS wash, the slides were wiped, and the encircled tissue overlaid with fluorescein isothiocyanate-conjugated goat antirabbit IgG (Cappel Laboratories, Cochranville, PA). After a 30 min incubation at 37°C, the slides were washed again in PBS, dried, mounted utilizing glycerol with paraphenylenediamine [100 mg *p*-phenylenediamine in 10 ml PBS and 90 ml glycerol with final pH adjusted to 8·0 with 0·5 M carbonate-bicarbonate buffer pH 9·0 (Johnson & de C Noqueira Araujo, 1981)], coverslipped, and sealed with clear nail polish. After studying and photographing immunofluorescence findings, the slides stained with anti-MBP were counterstained with haematoxylin and eosin (H & E) to identify the morphological relationship between immunofluorescent findings and the histopathological features of the disease.

The specificity of MBP localization by immunofluorescence has previously been investigated in detail (Filley *et al.*, 1981; 1982). Non-specific staining of eosinophils due to the acid–fluorone dyes is abolished by incubation in chromotrope 2R (Johnston & Bienenstock, 1974; Filley *et al.*, 1981; 1982). Controls were performed by staining a serial section with purified rabbit IgG in each case. In addition, serial sections of biopsies from individuals with normal skin and serial sections of biopsies from patients with dermatoses were stained with rabbit IgG and anti-MBP. In particular, because of the possibility that immunoreactive anti-MBP might stain the flame figures in lesions of eosinophilic cellulitis due to the apparently altered nature of the collagen in these areas, biopsies from dermatoses with prominent necrobiosis were tested as disease controls, including granuloma annulare (six patients), necrobiosis lipoidica diabeticorum (four patients) and rheumatoid nodules (four patients).

Clinical histories

Patient 1. A 26-year-old woman presented in September 1978 with the complaint of blisters involving the feet. She was well until September 1977 when she developed a papulovesicular eruption over the right hand and forearm 2 days after cutting her right thumb and receiving erythromycin for presumed secondary infection. The eruption was associated with massive oedema of the entire right arm. Similar lesions subsequently developed over the left wrist and back. She improved somewhat on prednisone treatment for 2 months. In January and March 1978, she developed pruritic erythematous papulovesicles and pustules on the lower back and chest and she responded to a short course of prednisone. Four months later, after prolonged sun exposure, she noted erythema and oedema of the left thigh and swelling of the hands, which subsided after prednisone treatment. As the steroids were tapered, she developed blisters and ulcers of the left foot and an indurated plaque of the left posterior calf.

In September 1978 she had infiltrative papules and plaques of the left foot, trunk, left posterior calf, and left temple (Fig. 1). She had no systemic symptoms or abnormalities on general physical examination. Laboratory data included a maximum peripheral eosinophil count of 548/mm^3, and bone marrow biopsy showed eosinophilia. Two skin biopsies showed changes of Wells' syndrome. Direct immunofluorescence on fresh-frozen tissue from the left foot lesion was negative.

The disease was initially controlled with prednisone and sulphapyridine. Prednisone was tapered slowly and she did well until January 1979 when new lesions appeared on the right foot, controlled with increased doses of prednisone. She was subsequently managed with prednisone plus dapsone and now has no active disease (July 1982).

Patient 2. In April 1978 this 21-year-old woman developed pruritic annular erythematous lesions of the feet associated with bilateral foot oedema and left foot blisters. This was soon followed by development of oedematous plaques of the hands, with a vesiculobullous eruption of the wrists, and swelling of the left arm. In June 1978 the lesions appeared as tender, morphoea-like plaques, primarily over the abdomen, left posterior thigh, right shoulder, and right back (Fig. 2). She had no systemic complaints and general exam was negative. Maximum peripheral eosinophil count was 3009/mm^3. A chest roentgenogram showed a lobulated mass in the left upper lung which was diminishing on serial films obtained over the next month, suggesting a resolving infection. Sputum samples showed no eosinophils, and PPD was negative. Two skin biopsies were obtained for histopathology and demonstrated the features of eosinophilic cellulitis. Direct immunofluorescence on two fresh-frozen specimens was positive for IgM in a granular pattern along the basement membrane.

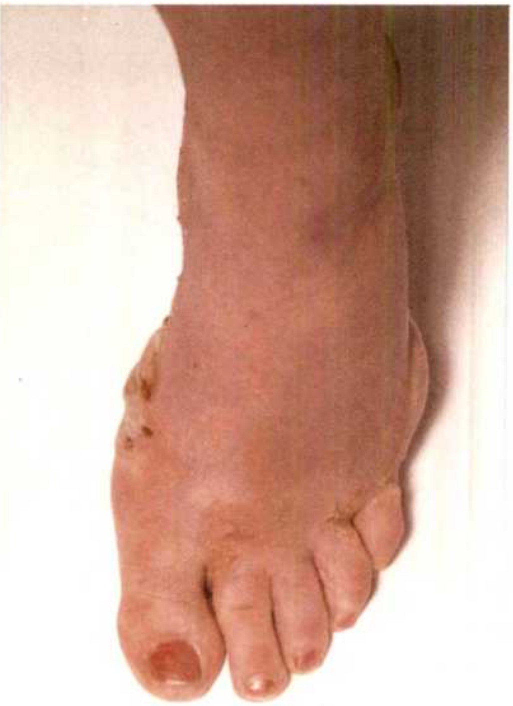
TABLE 1. Localization of MBP in Wells' syndrome

Patient	Age	Sex	Blisters associated with lesions	Direct immunofluorescence	Maximum peripheral eosinophils/mm^3	Immunofluorescence staining of eosinophils and flame figures with anti-MBP	Associated problems
1	26	F	+	—	548	+	None
2	21	F	+	Basement membrane, granular staining with IgM	3009	+	Abnormal chest roentgenogram, possible infection
3	66	F	−	Vessels with IgA, properdin, and fibrinogen	2346	+	None
4	56	F	−	Not done	1406	+	Raynaud's disease, leukaemia

One month later, these lesions were markedly improved, though she had developed five additional small plaques. As of July 1982 she had no lesions.

Patient 3. In June 1977, a 66-year-old woman presented with a 1-month history of onset of an erythematous lesion of the right forearm, with a central 'bite mark', spreading over 24 h to involve the entire forearm, associated with swelling, heat, and tenderness. Two days later, she developed erythematous infiltrative lesions scattered over the trunk and extremities, which resolved leaving hyperpigmented macules. She gave a history of similar lesions occurring 5 years previously. The right arm swelling was associated with pain and stiffness of the right elbow, wrist, and metacarpophalangeal joints, with swelling and numbness of the fingers. There was swelling, erythema, induration and warmth from the wrist to 5 cm above the elbow. Physical examination was otherwise negative. Laboratory data included a maximum peripheral eosinophilia of 2346/mm^3, erythrocyte sedimentation rate 53 mm/h, negative anti-nuclear antibody, and normal serum complement studies (CH50, C3, C4). Roentgenographic examination showed an ill-defined mass extending along the medial aspect of the forearm. Deep biopsy with surgical exploration was performed and showed massive eosinophilia, plasma cells, histiocytes, and scattered flame figures in the dermis. Direct immunofluorescence was positive for fibrinogen staining of small fascial vessels, IgA in small vessels of muscle, and properdin in dermal blood vessels.

Patient 4. A 56-year-old woman give a history since 1967 of pruritic and painful urticarial plaques involving the waist, trunk and thighs, each lasting days to months. She gave a history of flares of lesions related to administration of a variety of drugs, including xylocaine, carbocaine, valium, and penicillin. She also had Raynaud's disease involving the feet. On initial presentation in December 1971, she had violaceous, indurated, poorly demarcated lesions of the neck, axillae, and forehead up to 10 × 5 cm. Maximum peripheral eosinophil count was 1406/mm^3. Two biopsies were obtained for histopathology, and the diagnosis of Wells' syndrome was made. Re-evaluation in 1972 was essentially unchanged and a bone marrow biopsy showed eosinophilia. Follow-up in 1977 revealed that she had had two episodes of lesions over the previous 2 years; laboratory data in 1977 included white blood cell count of 8900 with 3·5%



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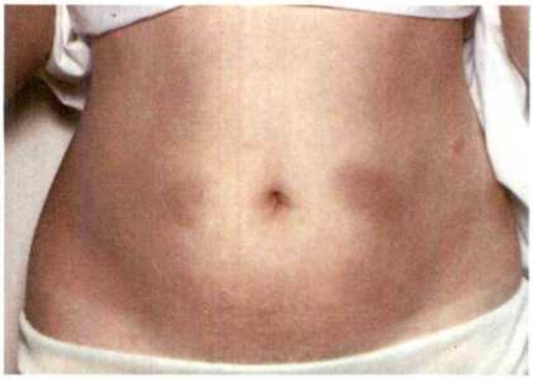

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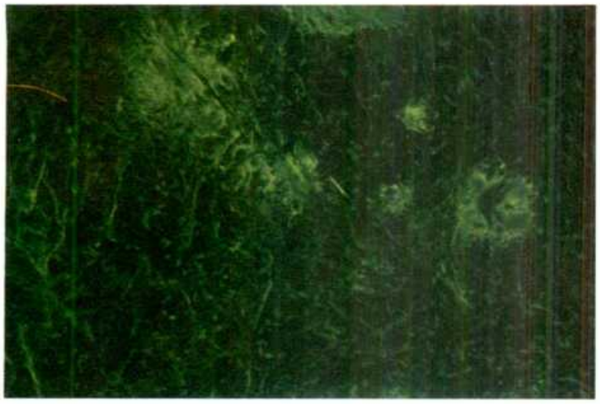

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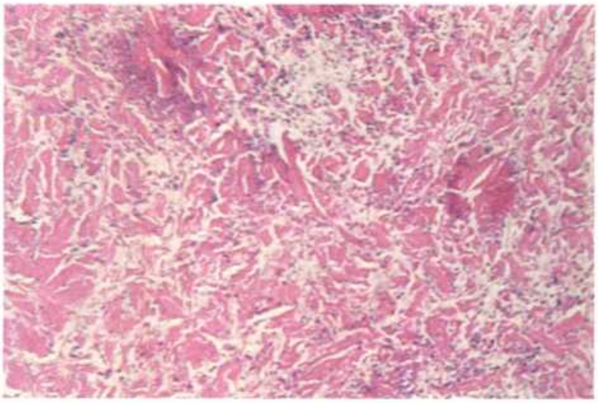

3b

FIGURE 1. Eosinophilic cellulitis (patient 1) showing infiltrative plaques associated with blisters.

FIGURE 2. Eosinophilic cellulitis showing a typical morphoea-like plaque (patient 2).

FIGURE 3. Photomicrographs comparing dermatopathology with immunofluorescence findings (× 100). (a) This anti-MBP stained section has fluorescence of several flame figures correlating with those seen after (b) counterstain of the same section with H & E. Control serial section stained with Staphylococcal protein A purified rabbit IgG was negative.

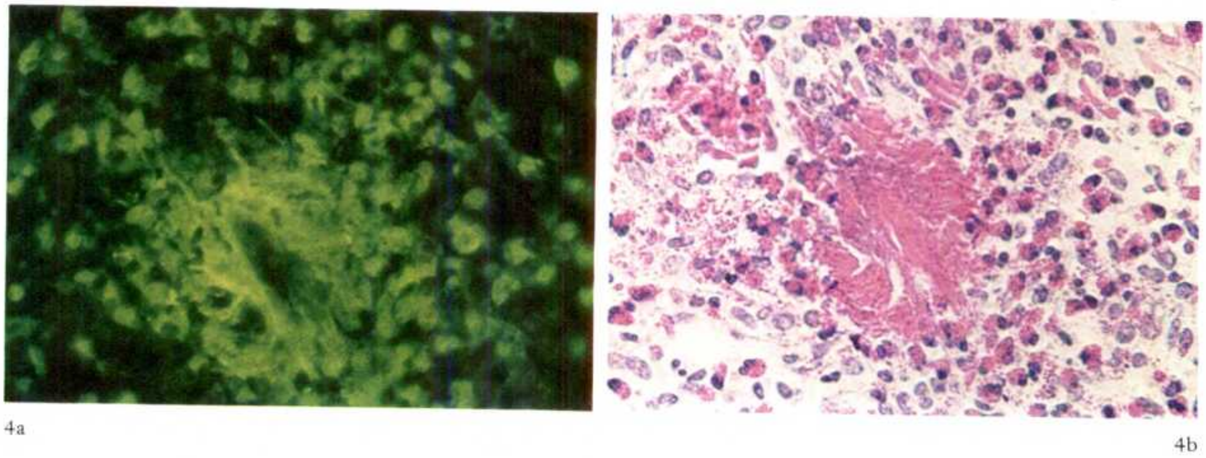

FIGURE 4. Photomicrographs showing an early flame figure (X 400). (a) Anti-MBP stained section shows fluorescence of numerous eosinophils adjacent to an early flame figure. The control serial section showed no fluorescence. (b) H & E counterstain of the section shown in (4a) clearly shows the eosinophils.

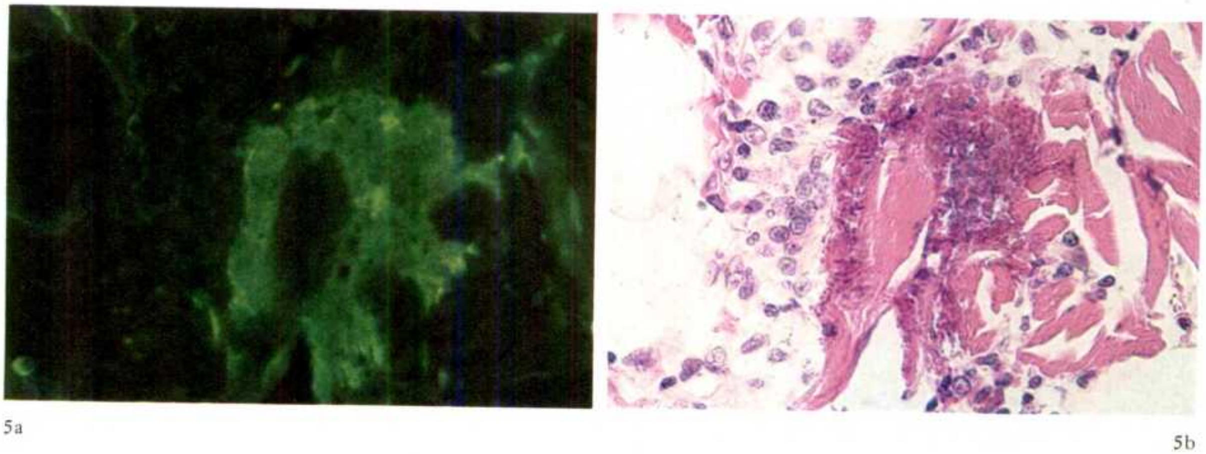

FIGURE 5. Photomicrographs illustrating a well-formed flame figure (X 400). (a) Anti-MBP stained section shows intense immunofluorescence of the flame figure, a central band of morphologically normal collagen which does not fluoresce, and no cellular fluorescence. The control serial section showed no fluorescence. (b) The H & E counterstain of the section shown in (5a) shows the well-formed flame figure surrounded by histiocytes.

eosinophils. In 1980, a bone marrow biopsy performed by her local physician showed acute non-lymphocytic leukaemia with a peripheral leukocyte count greater than 100,000.

RESULTS

Dermatopathology

The typical features of recurrent granulomatous dermatitis with eosinophilia (eosinophilic cellulitis) were well illustrated by our patients (Figs 3b, 4b and 5b). In patient 1, one biopsy had subepidermal bullae with eosinophils as well as massive dermal eosinophilia. The second biopsy from this patient had the well-formed characteristic features of the disease; masses of eosinophils were present throughout the dermis and adjacent subcutaneous tissue, and there were collections of histiocytes as well as histiocytes palisading around the flame figures. The flame figures in this and the other cases were pleomorphic and exhibited basophilic as well as eosinophilic amorphous or granular staining qualities. In general, very early evidence of flame figure formation could occasionally be seen in a few large fields of eosinophils (Fig. 4b), but well-formed figures were neither associated with nor surrounded by numerous eosinophils (Fig. 5b). The biopsy from patient 3 was similar to the first biopsy from patient 1 showing a predominance of tissue eosinophils and occasional flame figures.

Indirect immunofluorescence

Sections stained with affinity chromatography-purified anti-MBP had intense fluorescence compared with control sections stained with rabbit IgG. A precise morphological correlation between positive extracellular immunofluorescence of foci of dermal connective tissue and the presence of flame figures was seen in all patients (Figs 3a–5b). The pattern of staining with immunoreactive anti-MBP corresponded to the size and shape of each flame figure as verified by counterstain with H & E. Eosinophils also fluoresced brightly on sections stained with anti-MBP as compared with control slides (Fig. 4). Thus, one could more easily detect that well-formed flame figures were generally not associated with adjacent eosinophilia, but rather with a palisade of histiocytes (Fig. 5). The morphological variations of multiple flame figures in different stages of development in each biopsy were more readily identified by immunofluorescence than by H & E. MBP immunofluorescence was associated only with areas of apparently altered connective tissue and not morphologically normal collagen. The altered connective tissue appeared amorphous/granular, eosinophilic and basophilic. In the midst of each intensely fluorescent flame figure is a non-fluorescent band (Fig. 5a), representing a morphologically normal band of collagen, as defined by counterstain with H & E.

The basophilic amorphous and granular staining quality of segments of most flame figures is highly suggestive of necrobiosis, though the condition of the collagen in these foci is unknown. To control for the possibility that anti-MBP might preferentially adhere to altered collagen, we performed this immunofluorescence assay on biopsies from patients with granuloma annulare, necrobiosis lipoidica and rheumatoid nodules (fourteen patients total). We found that necrobiotic foci in these sections did not stain with anti-MBP. Furthermore, biopsies from four healthy volunteers with normal skin had no fluorescence when stained with anti-MBP.

DISCUSSION

Wells has described twelve cases demonstrating the typical clinical picture of recurrent episodes of oedematous, infiltrative plaques with a distinctive histological picture (Wells, 1971; Wells &

Smith, 1979). The acute stage was termed eosinophilic cellulitis, while older lesions were described as granulomatous dermatitis with eosinophilia, though both terms are generally considered to represent Wells' syndrome. There were equal numbers of male and female patients in Wells' series of twelve cases, while all of our patients were women. Our cases resemble those previously described in clinical course and morphology. Two of our four cases had blistering as part of the disease, as did five of the twelve reported by Wells. In one patient, morphoea-like plaques were the predominant clinical lesion, and Wells described this as a feature of resolving lesions in his patient 1. Table 1 summarizes the clinical, indirect and direct immunofluorescence features of our four patients. The association of Raynaud's disease and subsequent development of leukaemia in our patient 4 is unique and suggests that these patients need to be followed closely. All patients displayed peripheral eosinophilia, as was noted in six of Wells' cases. This is of particular interest in the light of studies showing that eosinophils from patients with peripheral eosinophilia are 'activated' (compared with eosinophils from normal controls) with reference to surface charge, lysosomal acid phosphatase, membrane hexose transport, and the hexose monophosphate pathway (Bass *et al.*, 1980). Furthermore, serum levels of MBP are elevated in a large group of patients with peripheral eosinophilia (Wassom *et al.*, 1981). Administration of systemic corticosteroids seems to be beneficial in Wells' syndrome, possibly through their ability to produce blood eosinopenia as well as decreased eosinophil chemotaxis (Altman *et al.*, 1981).

All biopsies showed prominent extracellular deposition of MBP in flame figures as well as intracellular localization of MBP to eosinophils. Controls consisting of serial sections stained with normal rabbit IgG, sections of skin from healthy volunteers, and biopsies from patients with necrobiotic lesions failed to show extracellular MBP deposition. The finding of extracellular fluorescence of MBP throughout all flame figures implies that the MBP is pathogenically related to the formation of these figures by binding to and altering collagen. Absence of MBP fluorescence of necrobiotic foci in biopsies from patients with diseases such as granuloma annulare demonstrates that extracellular fluorescence of MBP in flame figures is not due to non-specific staining of altered collagen. Furthermore, though the basophilic amorphous quality of many flame figures suggests focal necrobiosis of connective tissue, it is not known whether these figures represent structural alteration or merely morphological alteration due to coating of collagen. Our results show that very early flame figures are associated with marked adjacent tissue eosinophilia, while the well-formed flame figures are associated with few or no distinct intact eosinophils. This suggests that the eosinophil degranulates and flame figures form in association with foci of degranulation, and that a histiocytic infiltrate palisading around these foci of apparently altered connective tissue then follows as the third stage. Whether MBP is the mediator of this process or merely serves as an immunofluorescent marker for the patterns of eosinophil degranulation and dispersion of other mediators remains to be determined. That the histology of some reported cases of toxocariasis resembles flame figures, as pointed out by Wells and Smith (1979), is of significance in view of the known cytotoxic properties of MBP against parasites. Because extracts of rat eosinophils contain at least as much collagenase as neutrophils (Bassett *et al.*, 1976), it is also tempting to suggest a role for eosinophil collagenase in the apparently necrobiotic flame figures of Wells' syndrome, though evidence is lacking.

This indirect immunofluorescence technique provides a means of retrospectively analysing skin biopsies for the presence of eosinophil granule MBP, and thus a method of correlating the pathological changes with patterns of eosinophil degranulation in lesions of Wells' syndrome. The data suggest that eosinophils, through action of the cytotoxic MBP, mediate the tissue injury resulting in the infiltrative, oedematous, erythematous plaques that characterize this syndrome. The recurrent non-scarring nature of Wells' syndrome suggests that the major morphologic abnormality, namely flame figures, represents binding of MBP to connective tissue foci, with subsequent alteration of normal function rather than local tissue destruction.

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Hyperactivity of fibroblasts cultured from psoriatic skin: I. Faster proliferation and effect of serum withdrawal

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SUMMARY

Fibroblasts were cultured from the involved and uninvolved forearm skin of patients with severe generalized psoriasis and compared with those from the forearms of normal controls of similar ages. Thirteen strains were obtained from involved skin (PSA strains) and sixteen strains from uninvolved skin (PSB), with thirteen control strains (NSF).

Outgrowth of fibroblasts from the psoriatic skin explants was slightly quicker than from control skin and the average proliferation rates of passaged strains were PSA 144, PSB 134 and NSF 94 ($P < 0{\cdot}05$). Psoriatic fibroblasts were abnormally dependent on serum for anchorage. In serum-free medium many cells rounded up and were only loosely attached to the substratum. This effect was rapid, reversible and not corrected by adding fibronectin. Cell attachment assays showed only small differences between the psoriatic and normal fibroblasts and the main effect of serum withdrawal appeared to be on spreading rather than attachment.

These data suggest that the dermis of both involved and uninvolved psoriatic skin is abnormal and that the hyperactivity persists *in vitro*. Our findings seem most compatible with a hyperproliferative reaction of both epidermis and dermis to an extracutaneous, perhaps vascular, stimulus.

The conspicuously increased turnover of epidermis in psoriasis has diverted attention away from several hints of abnormality in the dermis. Dilated tortuous capillaries are a familiar histological feature but there is neglected evidence of less cross-linked (and therefore less stable) collagen (Horvath & Tabora, 1972), of a greater content of glycosaminoglycans (Fleischmajer & Blumenkrantz, 1970), and of increased urinary excretion of the mesenchymal catabolites, hydroxyproline and uronic acids (Brunish & Sorensen, 1965). The requirement for the dermal component in the Köbner phenomenon has been demonstrated (Stankler, 1969) and survival of the psoriatic phenotype in skin transplanted to nude mice required the presence of psoriatic dermis (Briggaman & Wheeler, 1980). Fibroblasts resident in the dermis are responsible for

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maintaining its substance and for many of its properties, normal or abnormal. Cell culture allows us to examine the metabolism of these cells under controlled conditions and in great detail, with minimum inconvenience to the patient. In this paper we compare the proliferative properties of fibroblasts cultured from the involved and uninvolved skin of patients with psoriasis with those of fibroblasts from normal subjects: a second paper will describe macromolecule synthesis by the same cell strains.

METHODS

Selection of patients

Patients were chosen from those attending this Department as in-patients for treatment of generalized plaque psoriasis (affecting more than 20% of their skin surface). All were males, without arthropathy and none had recently used topical or systemic corticosteroids. Prior to treatment 5-mm punch biopsies of full thickness skin were taken from the forearm. Informed consent was obtained. Uninvolved skin was sampled adjacent to the lesional biopsy site. The control subjects were mostly hospital staff, twelve males and two females, with no evidence of skin disease; 4-mm punch biopsies were taken from forearm sites. Patients and controls had a similar range of ages, approximately 20–70 years, with mean values of 40 ± 3 (s.e.m.) and 38 ± 4 respectively.

Isolation of cell strains

Skin biopsies were soaked in Dulbecco-Eagle medium containing 100 units/ml nystatin, 500 units/ml penicillin, 50 μg/ml streptomycin and 4 mM glutamine (all obtained from Gibco Europe, Paisley, Scotland) for several hours. After further washing they were chopped into 1-mm fragments and arranged beneath glass coverslips in plastic dishes. Medium containing 20% fetal calf serum (Gibco), 100 units/ml penicillin, 100 μg/ml streptomycin and 4 mM glutamine was added and the cultures were gassed with 5% CO_2/95% air for incubation at 37°C.

When a sufficient growth of fibroblasts had appeared, usually after about 4 weeks, the cells were removed with trypsin–versene and transferred to a Nunc plastic flask (25 cm^2 growth area) in medium with 10% fetal calf serum. Weekly subcultures were made and stocks of low passage cells were stored in liquid nitrogen. Strains derived from involved psoriatic skin were designated PSA, those from uninvolved skin PSB and those from control skin NSF.

Proliferation tests

Proliferation was assessed in early passage (3rd to 7th) cultures as in previous work (Priestley & Brown, 1980). For each strain ten flasks were seeded with aliquots of about 10^5 cells (day 0). On day 1 media were renewed. On day 3 five flasks were used for cell counts: cell suspensions were prepared with trypsin–versene and counted in an electronic counter (Coulter Model DN). Media in the remaining flasks were renewed on days 3 and 4 and the cells were harvested for counting on day 6. The increase in cell number between days 3 and 6 was expressed as a percentage of the average day 3 total.

Serum dependence and cell attachment assay

With some of the first cell strains to be established it was observed that washing in serum-free medium or phosphate-buffered saline (Dulbecco A) caused some cells to round up. Transfer to serum-free but otherwise complete medium then became a standard challenge to all cell strains. Cultures were incubated at 37°C and inspected every 5 min and then at longer intervals up to 16 h.

In some instances several concentrations of serum were used in different, coded flasks and the results were read by an observer without knowledge of the code. Human fibronectin (Nova Biochemicals) was added at 10 μg/ml either before or after cell rounding to test for prevention or reversal of the effect.

Cell attachment assays were performed by a modification of the method of Klebe (1974). In each assay a psoriatic and a control fibroblast strain were compared. Cell suspensions were prepared and washed well in serum-free medium; aliquots of cells were then plated in specially prepared 24-well dishes while other aliquots were counted to establish the number of cells used. The plates were prepared by pre-incubation in medium containing 0, 0.1, 1, 2 or 10% serum. After washing in Dulbecco A the plates were next incubated with 0.2% heat-denatured bovine serum albumin for 1 h, followed by a further washing. The cells were then allowed to attach for 3 h at 37°C in the atmosphere of 5% CO_2/95% air, after which the plates were gently washed free of non-adherent cells. The attached cells were then removed with trypsin–versene and counted for comparison with the initial aliquots.

RESULTS

A total of twenty-nine fibroblast strains from psoriatic skin (thirteen PSA from the involved skin and sixteen PSB from uninvolved skin) were established, including pairs of A and B strains from eight patients. Fourteen NSF strains were available as controls.

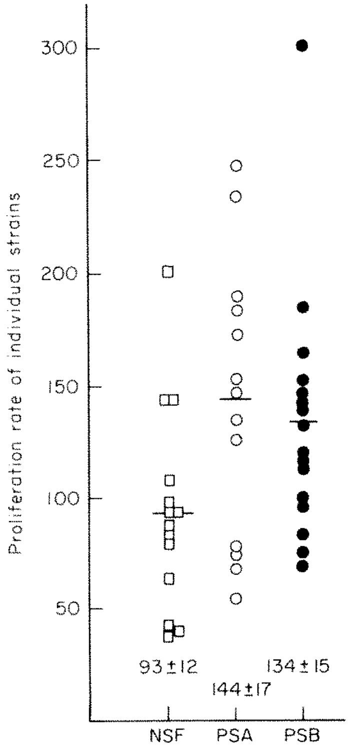

FIGURE 1. Proliferation rates of individual fibroblast strains. Group means are indicated by bars and shown below with their standard errors. PSA = fibroblasts from involved psoriatic skin; PSB = fibroblasts from uninvolved psoriatic skin; NSF = control fibroblasts.

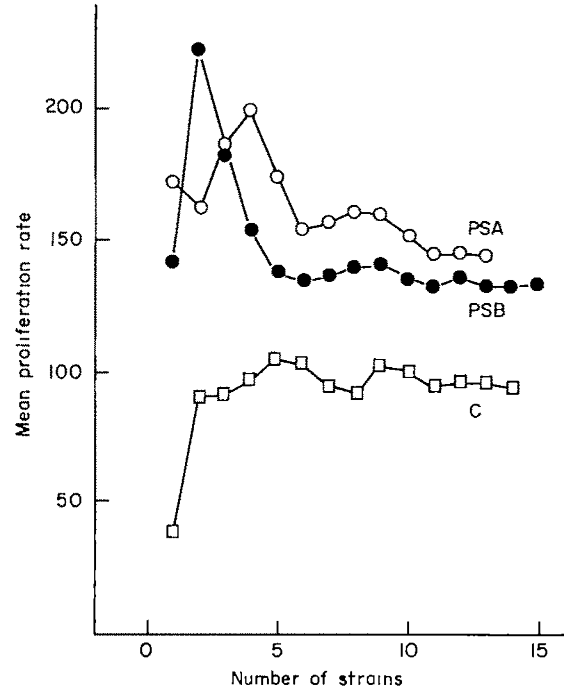

FIGURE 2. Mean proliferation rates as further cell strains were assessed. The values were stable after n = 5.

While isolating the cells we gained the impression that the psoriatic fibroblasts became established slightly quicker than control cells. This first suggestion of greater activity was tested retrospectively by tabulating the times from biopsy until the outgrowth was dense enough to permit a first passage. The average times (± s.e.m.) were PSA 27 ± 3, PSB 28 ± 2, NSF 35 ± 2 days and the difference of about 1 week was statistically significant (*t* test; $P < 0{\cdot}05$).

In proliferation assays each group gave a wide scatter of values (Fig. 1), but the mean values for PSA and PSB were both significantly different from the controls (*t* test; $P < 0{\cdot}05$). The PSA strains averaged 55% faster proliferation and PSB 44% compared with NSF. Once a small number (n = 5) of strains of each type has been assembled (Fig. 2) the mean values were remarkably stable and the differences between psoriatic and controls showed little change as further strains were added. In the eight paired PSA and PSB strains the mean proliferation values (± s.e.m.) were PSA 157 ± 18% and PSB 146 ± 25%, against a mean of 92 ± 20% in eight NSF strains matched for age of donor ($P < 0{\cdot}05$ for PSA using Wilcoxon's test for paired samples), again showing that fibroblasts from uninvolved skin resemble those from the involved skin rather than those from normal skin.

When the standard medium containing 10% fetal calf serum was replaced by serum-free medium a proportion of the fibroblasts in cultures of psoriatic cells rounded up and some cells detached when the flasks were moved. The effect was rapid (Fig. 3) and progressive and could be reversed by replacing the serum. It did not occur in medium containing 1% serum. The proportion of cells participating in the effect varied between different strains with a maximum of about one third (Fig. 3). All twenty-eight PSA and PSB strains reacted in this way but only two NSF strains. Fibronectin added to serum-free medium at 10 μg/ml neither prevented nor

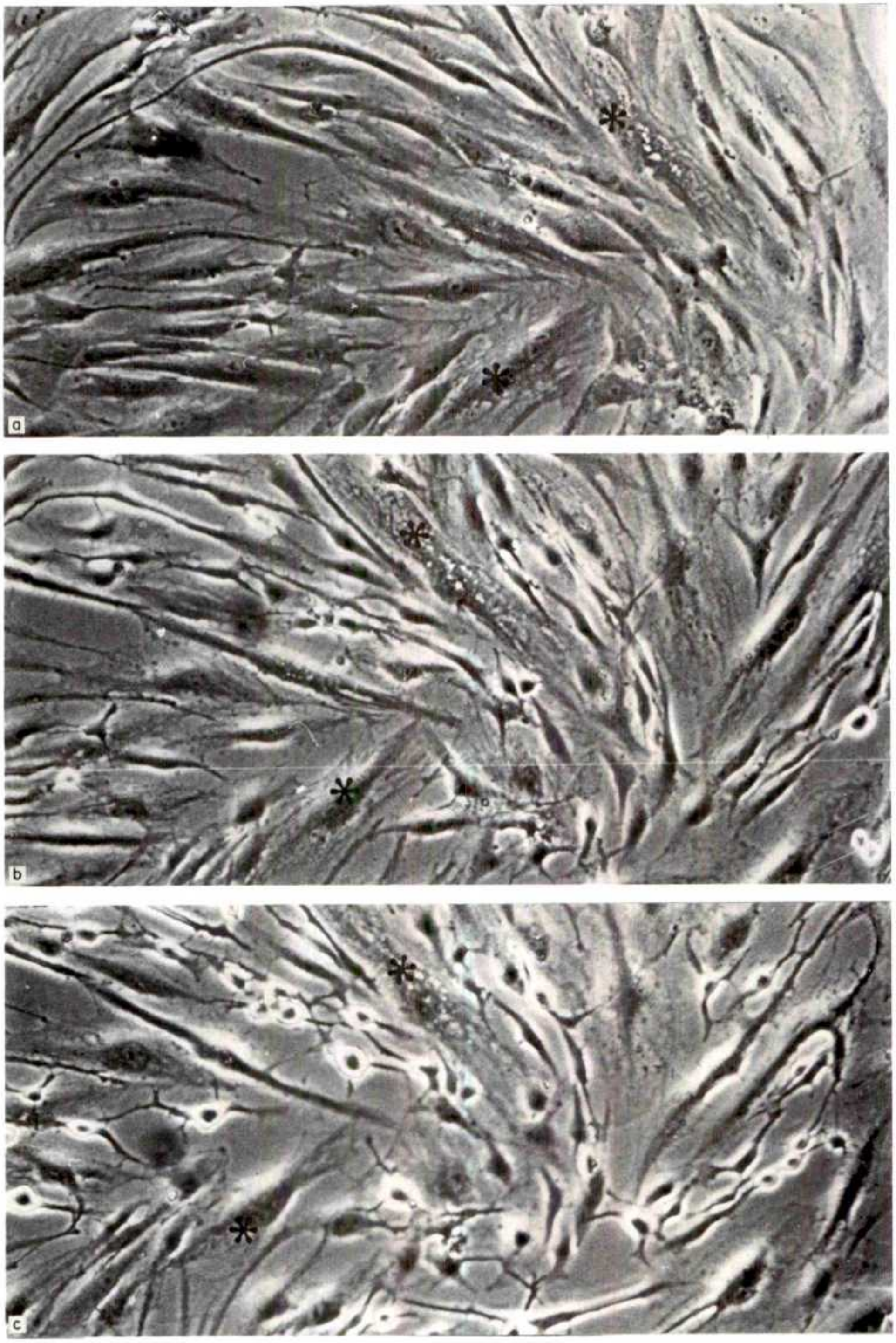

FIGURE 3. Effect of serum-withdrawal on psoriatic fibroblasts. (a) normal morphology in complete medium; (b) 20 min after substitution with serum-free medium; (c) after 60 min. The field was identified by two distinctive cells (asterisks), (phase-contrast, × 350).

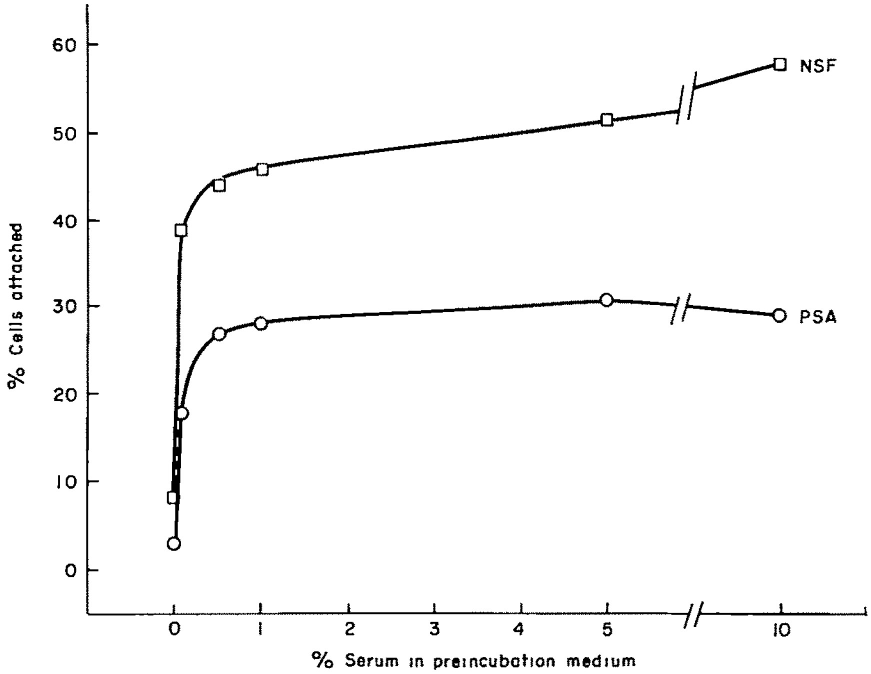

FIGURE 4. Attachment of psoriatic (PSA) and control (NSF) fibroblasts to plastic dishes prepared by preincubation with various percentages of fetal calf serum.

reversed the reaction; a 50% increase in the concentration of Ca^{2+} and Mg^{2+} was similarly ineffective.

Comparisons of the attachment of psoriatic and normal fibroblasts gave inconsistent results, from clearly superior attachment by the normal cells (Fig. 4) to similar or poorer attachment by the normal cells. However, tabulation of the data produced lower mean values for cell attachment in the psoriatic strains in 2, 5 or 10% serum. Mean values after preincubation in 2% serum were PSA 13% (n=4), PSB 13% (n=4) and NSF 28% (n=7), but the ranges were wide and the differences were not significant. This is compatible with the appearance of the cells in serum-free medium where the main effect was contraction rather than detachment.

DISCUSSION

The cellular hyperactivity in psoriasis has been regarded as an epidermal phenomenon (Shuster, 1971). These experiments show that cells resident in the psoriatic dermis have abnormally high rates of proliferation when maintained in culture and those from the uninvolved skin are almost as active as those from the lesion.

One possible explanation for these findings is that the hyperactivity of the psoriatic fibroblasts reflects changes in the geometry of the dermis in this disease: a greater proportion of papillary as opposed to reticular dermis might yield different ratios of papillary and reticular fibroblasts, which have different rates of growth *in vitro* (Harper & Grove, 1979). Although it has not been demonstrated that the proportion of papillary dermis does increase, it is credible that more papillary fibroblasts, which have the faster growth rate, might be derived from lesional skin in this manner to explain the higher growth rate of the PSA strains. The hypothesis does not explain, however, the increased proliferation of PSB strains from uninvolved skin with virtually normal histology. Furthermore, according to the most recent report (Tajima & Pinnell, 1981) the papillary and reticular fibroblasts have identical rates of collagen synthesis, whereas in both PSA and PSB strains of fibroblasts collagen synthesis was increased (Priestley, 1983). A shift in the ratio of papillary and reticular fibroblasts would not, therefore, account for our findings.

Observations on fibroblasts within the skin are so difficult that we have little information on their numbers and we cannot compare their proliferation *in vitro* and *in vivo*. However, the increased urinary excretion of glycosaminoglycans and of depolymerized uronic acids in these (Priestley, 1981) and other psoriatic subjects (Brunish & Sorensen, 1965; Mier & Urselmann, 1970), and of hydroxyproline (Brunish & Sorensen, 1965), point to increased metabolism in the dermis and in other connective tissue, compatible with hyperactivity of dermal cells.

We conclude that in psoriasis the entire skin is abnormal: the epidermis and the dermis of both involved and uninvolved areas are hyperactive, and the stimulus to hyperactivity or its effect persists in dermal cells *in vitro*. A similar survival of the abnormality is seen in psoriatic skin transplanted to nude mice (Kreuger, 1981) and in epithelial outgrowths from psoriatic skin explants *in vitro* (Harper *et al.*, 1978).

We have found that medium transferred from cultures of psoriatic fibroblasts to normal skin epithelial cells did not stimulate their proliferation (Priestley & Adams, 1983) in agreement with the findings of Baden, Kubilus & MacDonald (1981). It seems unlikely that fibroblasts have a primary role in pathogenesis and neither do we regard fibroblast hyperactivity as secondary to events in the epidermis, since those from the uninvolved skin were equally abnormal. Fine differences in the timing of epidermal and dermal pathology during the development of lesions (Braverman & Sibley, 1982) may merely reflect different reaction times after a common stimulus and we consider our findings are most compatible with a hyperproliferative reaction by both epidermis and dermis to an extracutaneous stimulus, probably transmitted vascularly (Mali, 1979). There is ample evidence of abnormality in peripheral blood and in inflammatory cells within psoriatic skin (Gelfant *et al.*, 1982) and elimination of polymorphonuclear leukocytes has been suggested as the mechanism of successful peritoneal dialysis therapy (Glinski *et al.*, 1980).

An unusual dependence on serum for anchorage in the psoriatic fibroblasts was observed but not explained in this work. Since our first preliminary report of this feature of the cells (Priestley, 1981) the same behaviour has been noticed in psoriatic strains isolated elsewhere (I.M.Leigh, personal communication). In transformed cells or those derived from tumours loss of proliferative control is accompanied by deficient synthesis of the cell surface glycoprotein fibronectin and altered adherence so that many cell lines will grow in suspension culture (Yamada & Olden, 1978). We were impressed by the parallel with our psoriatic fibroblasts but were unable to show an increased requirement for fibronectin, and the abnormality seemed to involve spreading rather than attachment mechanisms. However, there is almost certainly more than one attachment factor (Harper & Juliano, 1981) and separate factors may control spreading and attachment (Chen & Singer, 1980).

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Hyperactivity of fibroblasts cultured from psoriatic skin: II. Synthesis of macromolecules

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SUMMARY

Glycosaminoglycans and protein synthesis were assessed in fibroblasts cultured from the involved (PSA strains) and uninvolved (PSB strains) forearm skin of patients with severe generalized psoriasis and compared with that in fibroblasts from the forearm skin of control subjects of similar ages (NSF strains).

Average rates of glycosaminoglycan secretion were 19% (PSA) and 28% higher (PSB) in psoriatic strains. Although these differences were not significant in themselves, the relationship between glycosaminoglycan secretion and fibroblast density (cells/cm^2) was significantly different ($P = 0{\cdot}02$) for the psoriatic and control groups, with the psoriatic cells secreting appreciably more glycosaminoglycans at higher cell densities. The nature of the glycosaminoglycans produced was the same in psoriatic and normal strains; 90% was recovered in the culture medium and the bulk of this appeared to be hyaluronic acid with only small amounts of sulphated components. Collagen synthesis was 77% higher in PSA and PSB strains ($P < 0{\cdot}01$), while other protein synthesis was increased by 44% ($P < 0{\cdot}05$).

These findings confirm that fibroblasts from psoriatic skin are hyperactive *in vitro*. The data are compatible with the results of studies of psoriatic skin *in vivo* and suggest that the entire skin is abnormal.

In a previous paper (Priestley & Adams, 1983) we described the isolation and culture of fibroblasts from the involved and uninvolved skin of patients with severe plaque psoriasis. These fibroblasts proliferated faster than fibroblasts from matching sites on control subjects of similar ages to the patients. In this paper we describe comparisons of protein and glycosaminoglycans (GAG) synthesis in the same strains of normal and psoriatic fibroblasts.

METHODS

Thirteen fibroblast strains from the involved skin (PSA strains), fifteen strains from the

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uninvolved skin (PSB strains) and fourteen strains from normal subjects (NSF strains) were available (Priestley & Adams, 1983). The cells were maintained by conventional cell culture techniques in Dulbecco-Eagle medium with 10% fetal calf serum, glutamine, penicillin and streptomycin (Gibco–Biocult, Paisley, Scotland). Protein and GAG synthesis were assessed in cells passaged 4–8 times, most of which had been stored in liquid nitrogen for periods of weeks or months.

Glycosaminoglycans

Rates of GAG secretion were measured in culture media collected from the proliferation experiments (Priestley & Adams, 1983) and stored at −20°. Media added to small flask cultures (25 cm^2 growth area) on the fourth day were collected on the sixth day when the cells were counted. This material was processed by dialysis against sodium acetate buffer, concentrated with Lyphogel® polyacrylamide beads, digested with hyaluronidase and protein was precipitated in 5% trichloracetic acid (TCA) as previously described (Priestley & Brown, 1979). The uronic acid content was then measured colorimetrically using *m*-hydroxydiphenyl (Blumenkrantz & Asboe-Hansen, 1973) with a glucuronolactone standard. Unused culture medium was processed with each batch of samples and its GAG content was subtracted from that of the experimental samples. GAG outputs were expressed as μg uronic acid secreted per 10^7 cells. A reciprocal relationship between secretion and proliferation rate was analysed in terms of the final cell density in the cultures. If this had been very low (3×10^5 cells per flask) the experiments were repeated with higher initial cell seedings and only data from medium or high density cultures were used to compare mean rates of secretion in the psoriatic and normal strains.

Qualitative studies of GAG synthesis and secretion were made by incubating cells with [^{3}H]glucosamine and analysing the radioactive GAGs by cellulose acetate electrophoresis (Bashey *et al.*, 1977). Large cultures (about 2×10^6 cells) of three PSA, three PSB and three NSF strains were labelled for 24 h in 10 ml medium containing 100 μCi (3·7 MBq) D-[6-^{3}H]glucosamine hydrochloride (Amersham). The labelled cells were washed, released from the flasks with trypsin–versene and homogenized. GAG in the cell homogenates and in the media was then isolated by dialysis against phosphate buffer at 4°C, concentration with Lyphogel®, digestion with papain overnight and further dialysis. The GAG was then precipitated with 5% cetyltrimethylammonium bromide (1:30) and redissolved in 0·2 ml distilled water. Of this 10 μl were used for electrophoresis on cellulose acetate strips in 0·1 M copper acetate buffer (2 mA/cm for about 4 h). After electrophoresis the strips were stained in 0·1% Alcian Blue. The positions of the radioactive GAGs were deduced from the migration of hyaluronic acid, dermatan sulphate and chondroitin sulphate standards (Sigma) run on the side of each strip. The entire width of each strip encompassing the three standards was then removed or the entire strip was sliced into 2-mm sections for scintillation counting. A 2:1 mixture of NE233 scintillation fluid (Nuclear Enterprises, Edinburgh) and Triton X-100 was used and quench correction was by external standard channels ratio.

Protein synthesis

Collagen and other protein synthesis was assessed by incubating fibroblasts with [^{3}H]proline and distinguishing labelled collagen from other protein by its susceptibility to bacterial collagenase. The method was that used in earlier work (Priestley & Brown, 1979) except that 10% fetal calf serum was included during labelling because the adherence of psoriatic fibroblasts is affected in serum-free medium (Priestley & Adams, 1983).

Seven experiments were performed. In each a pair of PSA and PSB strains from the same patient were matched with an NSF strain from a donor of similar age. The cultures were established in multi-well plastic dishes so that each dish contained two cultures of each strain of fibroblasts. When confluent (about 2×10^5 cells per well) 50 μg/ml vitamin C was added for 2 days. Finally the cells were incubated for 24 h in medium containing vitamin C, 10% fetal calf serum, glutamine and 10 μCi (0·37 MBq) per well L-[5-^{3}H]proline (Amersham) but without antibiotics. The medium was then removed and the cells disrupted in distilled water. Material from the two identical wells in each dish were combined to give five samples of cells and five of medium for each strain. Protein was then precipitated with TCA, washed, dried and redissolved in Tris-HCl buffer pH 7·6, containing 10 mM N-ethyl maleimide and 10 mM calcium chloride. Each preparation was divided into two aliquots to one of which was added 100 units collagenase (Form III; Advance Biofactures Corporation, Lynbrook, U.S.A.). After incubation at 37°C for 16 h protein was precipitated in 5% TCA/0·25% tannic acid, washed and dried. The residues were dissolved in hyamine hydroxide and added to 10 ml NE233 scintillation fluid for scintillation counting. In each experiment cultures of each cell strain identical to those labelled with [^{3}H]proline were used for cell counts to allow expression of the results as dpm protein / 10^5 cells. Relative collagen synthesis was calculated by the method of Diegelmann & Peterkofsky (1972). All protein digested by the collagenase was regarded as collagen; although repeated tests in many laboratories have found this brand of enzyme to be completely free from other protease activity (Verbruggen, Salomon & Greene, 1981), others have controlled traces of protease with albumin and N-ethylmaleimide (Freiberger *et al.*, 1980).

RESULTS

The average rates of GAG secretion by the psoriatic fibroblasts were 19% (PSA) and 28% (PSB) higher than in controls (Table 1), but there was wide variation between the strains within groups and the differences were not statistically significant when compared directly. An important factor in this variation was the final cell density in the cultures, which itself varied according to the growth rate of the cell strain (despite the similar average densities in Table 1). The rate of GAG secretion was low in dense cultures of rapidly proliferating strains. Regression lines (Fig. 1) fitted to the combined data for PSA and PSB strains and to the data for NSF strains had significantly different gradients (−0·017 for PSA plus PSB; −0·057 for NSF; $P = 0·02$) (Snedecor & Cochrane, 1967), showing that at medium and high cell densities more GAG was secreted by the psoriatic strains.

The pattern of GAG types displayed by electrophoresis was similar in all nine fibroblast

TABLE 1. GAG secretion by psoriatic and normal fibroblasts

Fibroblast group	n	GAG ± s.e.m.	Mean cell density 10^5 cells/flask
PSA	13	95 ± 8	6·5 ± 0·7
PSB	14	102 ± 9	7·3 ± 1·0
NSF	13	80 ± 11	6·9 ± 1·1

n = number of strains assessed; GAG, μg uronic acid secreted/10^7 fibroblasts.

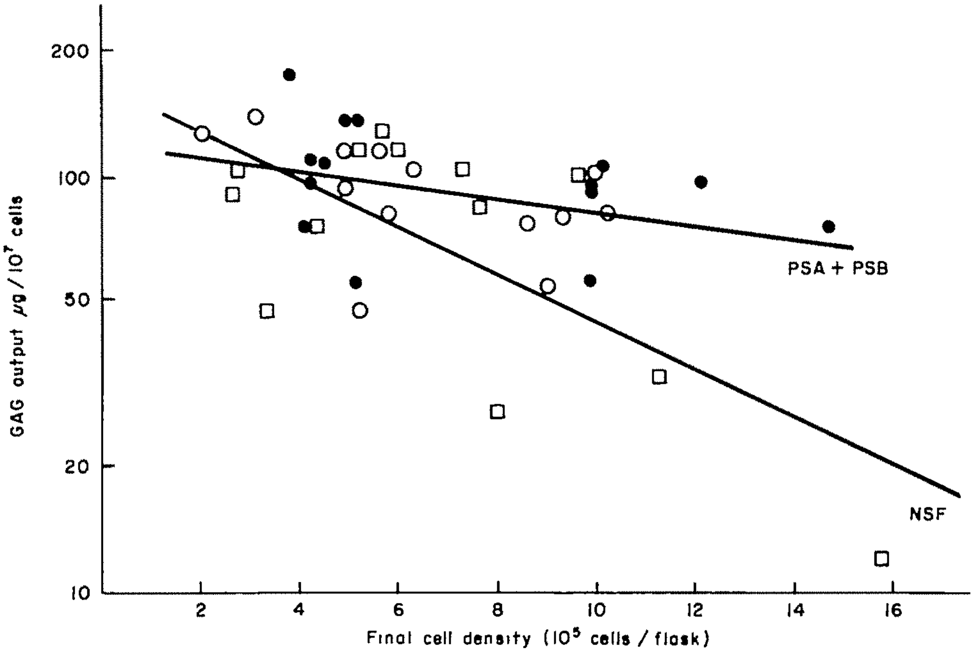

FIGURE 1. Semi-logarithmic plot of glycosaminoglycan secretion against cell density. Regression lines were calculated from the least mean squares and compared by the method of Snedecor & Cochrane (1967). For pooled PSA and PSB data (solid circles) $n=27$; $r=-0{\cdot}382$; $P<0{\cdot}05$. For NSF (square symbols) $n=13$; $r=-0{\cdot}695$; $P<0{\cdot}01$. The gradients differed significantly ($P=0{\cdot}02$).

strains examined. More than 90% of the radioactive GAG was recovered in the culture medium and of this the great bulk co-migrated with the hyaluronic acid standard (Fig. 2). Small amounts of material (corresponding to 4–7% of that present on each strip) co-migrated with the dermatan sulphate and chondroitin sulphate standards and an even smaller trace of radioactivity remained at the point of application, perhaps representing glycoprotein. The pattern of GAG in the cells appeared to be similar to that in the medium but the amount of putative sulphated (faster-migrating) material was barely above counting backgrounds because of the overall low level of radioactivity. Fig. 2 demonstrates that there was no disadvantage in merely using the Alcian Blue-stained portions of the strips for analysis rather than sectioning the whole strip into 2-mm sections.

The assessments of protein synthesis gave very clear results (Table 2). In all seven experiments the psoriatic fibroblast strains synthesized more collagen than the control strains and in six experiments other protein synthesis was also higher in the psoriatic strains. There was no consistent difference between the activities of strains from involved and uninvolved skin. In the group means, collagen synthesis was 77% higher in PSA and PSB strains while other protein synthesis was 44% higher than in controls. A selective increase in collagen compared to other protein synthesis is more apparent in these figures than in group mean values for relative collagen synthesis (Diegelmann & Peterkofsky, 1972) which were 5·7% in PSA and PSB against 4·5% in controls, a difference of 27% (n.s. by t test). The age of the donor of the skin appeared to have little influence on these results although unexplained differences in the efficiency of the labelling affected all the strains used in some experiments.

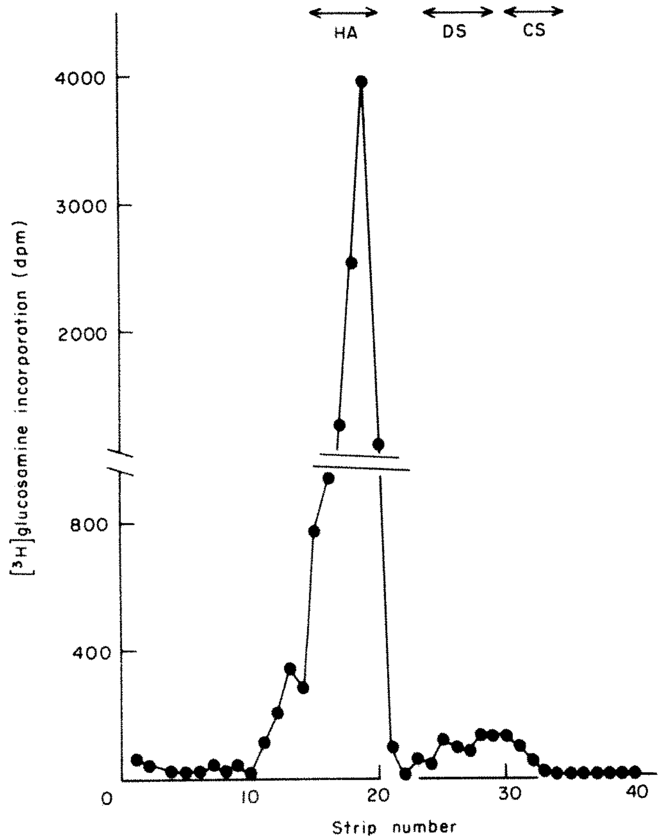

FIGURE 2. GAG components secreted by normal skin fibroblasts after [^{3}H]glucosamine labelling. Electrophoresis on cellulose acetate sheets was from left to right and the sheets were cut into 2-mm strips for scintillation counting. The position of stained standards (top) shows nearly all the material to be hyaluronic acid. Psoriatic fibroblasts gave similar patterns.

TABLE 2. Protein synthesis in psoriatic and normal fibroblasts *in vitro*

Experiment	Age of donor (y)	Collagen synthesis 1000 dpm/10^5 cells PSA	PSB	NSF	Other protein synthesis 1000 dpm/10^5 cells PSA	PSB	NSF
1	45	46	43	28	116	130	130
2	62	22	23	10	116	76	74
3	24	56	38	13	117	79	54
4	72	57	76	47	158	184	157
5	22	69	61	36	233	230	124
6	42	43	64	35	143	188	81
7	43	28	18	13	129	220	81
Mean ± s.e.m.		46 ± 6**	46 ± 8**	26 ± 5	144 ± 16*	144 ± 22*	100 ± 14

Each value is a mean from five determinations. Group means were compared using Friedman's two-way analysis of variance by ranks $^{*}P < 0{\cdot}05$ $^{**}P < 0{\cdot}01$.

DISCUSSION

In our three-way comparisons of protein synthesis, collagen synthesis was over 100% higher in some experiments in fibroblast strains from psoriatic skin. In overall terms the psoriatic strains synthesized 77% more collagen and 44% more other protein than strains from normal skin, a differential increase in collagen synthesis. The equivalence of the activities of PSA and PSB cells from involved and uninvolved skin strongly suggests that the greater collagen synthesis is not a repair response to the lytic actions of inflammatory cells in the involved dermis perpetuated *in vitro*.

These findings from cultured fibroblasts are compatible with earlier evidence of altered collagen metabolism obtained directly from patients. This included reports of increased propyl hydroxylase activity in lesions (Fleckman, Jeffrey & Eisen, 1973), of a more soluble collagen deficient in cross-links (Horvath & Tabora, 1972), of increased uptake of 99^{m} technetium diphosphonate in the joints of non-arthritic psoriasis patients (Namey & Rosenthall, 1976), and of increased urinary excretion of hydroxyproline (Brunish & Sorensen, 1965). Both synthesis and degradation of collagen in the psoriatic skin must increase to the same extent, so that the collagen content of the skin is unchanged. The difference between psoriatic and normal skin then resembles the difference between the skins of children and adults in terms of collagen metabolism, since collagen synthesis and hydroxyproline excretion are both several times higher in children (Uitto, 1970; Jasin *et al.*, 1962) without a notable difference in collagen content. This may be related to levels of growth hormone which influences both collagen synthesis and hydroxyproline excretion (Jasin *et al.*, 1962), compatible with the recent claim that in psoriasis high growth hormone levels persist after the second decade when levels in normal adults decline (Weber *et al.*, 1982). Both human growth hormone and its intermediary somatomedin stimulate fibroblast proliferation (Moon & St Vincent, 1957; Moses *et al.*, 1978), which was also increased in the psoriatic fibroblasts (Priestley & Adams, 1983).

Increased synthesis of GAG in psoriatic skin still seems likely. Interpretation of the 28% higher average rate of secretion in PSB fibroblasts and 19% higher rate in PSA fibroblasts was complicated by the decisive influence of cell density on the rate of secretion *in vitro* (Hronowski & Anastassiades, 1980) but synthesis by psoriatic fibroblasts was significantly greater at higher cell densities (Fig. 1). Reports of higher hyaluronic acid contents in psoriatic skin obtained *post mortem* (Fleischmajer & Blumenkrantz, 1970) were not confirmed in other work (Hollman *et al.*, 1971) where a 20% higher GAG content was not significant; larger samples of patients and controls might resolve such conflict. Epidermal GAG appears to decline in psoriatic skin (King, Tabiowo & Paul, 1981).

The question of GAG degradation in psoriasis is less contentious. There is support for an increase in urinary GAG (Brunish & Sorensen, 1965) although similar increases may occur in other skin diseases (Mier & Urselmann, 1965). Increased degradation would require increased synthesis to preserve the status quo. Our study of urinary GAG in these psoriasis patients also confirmed an increase but suggested that it might not originate in the skin lesions because, as in normal subjects, the pattern of GAG components was not characteristic of the skin (Priestley, unpublished).

The relative proportions of sulphated and non-sulphated GAG synthesized by the different fibroblasts *in vitro* were similar; normal and psoriatic fibroblasts secreted over nine times more hyaluronic acid than chondroitin sulphate and dermatan sulphate; a six-fold inequality *in vitro* was reported previously (Bashey *et al.*, 1977). The ratio approaches 1:1 in the skin content (Fleischmajer & Blumenkrantz, 1970) and the apparent over-production of hyaluronic acid may be partly an adaptation to *in vitro* conditions, although a two- to three-fold imbalance is necessary to compensate for hyaluronic acid's shorter half-life in the skin (Schiller *et al.*, 1956).

This study provides further evidence of abnormal dermal metabolism in psoriasis and invites speculation that other connective tissue may be affected even where frank arthropathy is not apparent (Namey & Rosenthall, 1976).

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Cutaneous factors related to the risk of malignant melanoma

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SUMMARY

In a case-control study, 287 women with malignant melanoma were compared with 574 age-matched controls. Red hair colour at age 5 years was associated with a tripling of risk [relative risk (RR) = 3·0], blonde hair with a 60% increase (RR = 1·6) and fair skin with a doubling (RR = 2·1). Women with melanoma also reported that they tended to burn (RR = 1·4) and to freckle (RR = 1·9) after exposure to sunlight. Since fair skin, red hair, and the tendency to burn or freckle after exposure to sunlight all cluster in the same individuals, the extent to which each of these factors had an independent influence on susceptibility to melanoma was investigated. Hair colour, especially red hair, proved to be the major determinant, followed by skin colour.

The reporting of above average numbers of naevi on the body was as strong a predictor of melanoma as was red hair colour (RR = 3·4). A history of psoriasis was also more common in cases than controls (RR = 3·0) as was a history of vitiligo (RR = 1·8). A history of acne appeared to be protective (RR = 0·4) as did a history suggestive of chloasma (RR = 0·6) and premature greying of the hair (RR = 0·6). These relationships were irrespective of hair and skin colour.

Malignant melanoma is classically described as being most frequent in fair-skinned, blue-eyed, red-haired persons who burn rather than tan on exposure to sunlight (McGovern, 1978). Although the relative rarity of melanoma in dark-skinned people is well recognized (Pack, Davis & Oppenheim, 1963) there have been surprisingly few studies in which various factors related to pigmentation have been compared in melanoma patients and suitably selected controls. Furthermore, the findings which have been reported are somewhat contradictory; in Australia, the U.S.A. and England, studies have noted that fair skin and hair colour were more common in persons with melanoma than controls (Lancaster & Nelson, 1957; Gellin, Kopf & Garfinkel, 1969; Adams *et al.*, 1981), but one study in Norway found no such relationship (Klepp & Magnus, 1979). The tendency to burn rather than tan on exposure to sunlight has been

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consistently found to be more common in melanoma patients than controls (Lancaster & Nelson, 1957; Gellin *et al.*, 1969; Adams *et al.*, 1981). Related to this, freckling after exposure to sunlight has also been associated with melanoma (Pack *et al.*, 1963; Klepp & Magnus, 1979). Since persons with a fair complexion are also those who burn and freckle after exposure to sunlight, it is not clear which is the major risk: actual colour or the way the skin reacts to sunlight.

The possible role of pre-existent skin lesions such as naevi (Milton, 1972; McGovern, 1977) or even vitiligo (Kopf, Bart & Rodriguez-Sains, 1977) is frequently discussed. In addition, the use of photochemotherapy for psoriasis has led to concern that in the long-term, skin cancer and possibly melanoma may be induced in a small number of those treated (Marks, 1982). No studies reported thus far have compared the frequency of naevi or skin conditions such as psoriasis in persons with melanoma and matched controls.

Data on 287 women aged 18 to 54 years with melanoma and 574 age-matched controls were examined in an attempt to establish which aspects of pigmentation or related factors were the most important determinants of melanoma.

SUBJECTS AND METHODS

Two hundred and eighty-seven Caucasian women aged 18 to 54 years with malignant melanoma were included in the study. All were treated in the Melanoma Clinic at Sydney Hospital between 1975 and 1980. Pathological diagnosis was confirmed by one pathologist, Professor V.J. McGovern. Details of the study have been described elsewhere (Beral *et al.*, 1982). The 287 women with melanoma and 574 age-matched controls were interviewed in their homes or at Sydney Hospital by trained interviewers using a standard questionnaire. It included questions on various demographic characteristics, sunbathing activities, reproductive history and other factors. Skin colour, eye colour and the presence of pigmented naevi on the body were assessed in two ways: by questioning and by independent grading by the interviewer. Since hair colour may change with age, each participant was asked about the colour of their hair when they were 5 years old and its colour at the time the melanoma was diagnosed (or at a corresponding time for the controls).

The completed questionnaires were coded and the data transferred to punch cards. Tabulations were obtained using a statistical computer package, XTAB, at the London School of Hygiene and Tropical Medicine. Relative risks (RR) and 95% confidence interval (CI) estimations, chi-squared tests of significance and Mantel-Haenszel adjusted relative risks were calculated using the methods described by Rothman & Boice (1979). Whenever the limits of the confidence interval (CI) do not include 1·00, the RR differs significantly from unity.

RESULTS

Hair, skin and eye colour were all related to melanoma risk. The presence of red hair in childhood was associated with a three-fold increase in risk compared with other hair colours (RR = 3·0, 95% CI 1·95 to 4·73) and blonde hair with a smaller increase (RR = 1·6, 95% CI 1·15 to 2·14). Fair skin, as opposed to medium or olive skin, was associated with an approximate doubling of risk (RR = 2·1, 95% CI 1·55 to 2·75). Compared with brown eye colour, the presence of blue eyes was associated with a small, non-significant increase in risk (RR = 1·2) and of green eyes with no increase in risk at all (RR = 1·0). Since all these factors are closely related, the distribution of various combinations of hair, skin and eye colours in the cases and controls, and the corresponding relative risks are shown in Table 1. Green and brown eye colour are

TABLE 1. Reported hair colour at age 5, skin colour and eye colour in cases and controls, and relative risk associated with each combination (data missing for three cases and six controls)

Hair colour	Skin colour	Eye colour	Cases	Controls	Relative risk†
Red	Fair	Green or brown	22	18	4·5**
Red	Fair	Blue	17	15	4·2**
Red	Medium or olive	Green or brown	10	11	3·4**
Red	Medium or olive	Blue	2	3	2·5
Blonde	Fair	Green or brown	33	48	2·6**
Blonde	Fair	Blue	48	63	2·8**
Blonde	Medium or olive	Green or brown	23	72	1·2
Blonde	Medium or olive	Blue	27	51	2·0*
Brown or black	Fair	Green or brown	28	56	1·9*
Brown or black	Fair	Blue	20	30	2·5**
Brown or black	Medium or olive	Green or brown	41	151	1·0
Brown or black	Medium or olive	Blue	13	50	1·0

* Differs significantly from 1·0, $P < 0{\cdot}05$.

** $P < 0{\cdot}01$.

† Relative to those with brown or black hair and medium or olive skin.

grouped together since there was little difference in risk associated with the two eye colours. All relative risks quoted are in comparison with women who reported black or brown hair and medium or olive skin colour. The main finding to emerge from Table 1 is the importance of red hair. Regardless of eye colour, red hair was associated with an approximate three- or four-fold increase in melanoma risk. Those with co-existing fair skin had a slightly higher risk (RR = 4·4) than did those with co-existing dark skin (RR = 3·2). After red hair second in importance was blonde hair. This in combination with fair skin was associated with an RR of 2·7 and in combination with dark skin with an RR of 1·5. Skin colour was also an independent risk factor, but its importance depended to some extent on the associated hair colour. For those with red hair, fair skin had only a small additional effect, increasing the risk by 39%; for those with blonde hair the risk was increased by 80%; but for those with black or brown hair the risk was increased by 108%. After adjusting for hair colour, fair skin was associated with an overall relative risk of 1·9 (95% CI 1·38 to 2·50). On the other hand, eye colour had no independent effect on risk.

Analysis of hair colour in adulthood was complicated by the differing percentage of cases and controls reporting that they had grey hair (5·2% and 6·3% respectively) or some greying of the hair (36% and 48% respectively). As is shown in Table 2, the apparent protective effect of greying of the hair was evident for all combinations of skin and hair colour at age 5 years, the

TABLE 2. Relative risk of melanoma associated with various characteristics, by hair and skin colour

	Hair and skin colour					All colours combined	
Characteristics	Red hair	Blonde hair and fair skin	Blonde hair and dark skin	Brown/black hair and fair skin	Brown/black hair and dark skin	Crude	Adjusted for hair and skin colour
Some grey hair versus none	0·4	0·9	0·7	0·6	0·7	0·6**	0·6**
Blistering or peeling after exposure to sunlight versus milder reactions	1·0	1·2	0·4	1·4	1·6	1·4*	1·1
Usually freckles after exposure to sunlight versus sometimes or never freckling	1·0	1·8	1·6	1·1	1·4	1·9*	1·4
Celtic versus non-Celtic origin (restricted to those born in Australia)	0·5	1·2	1·2	1·1	1·7	1·3	1·4
Above average numbers of naevi on the body versus average number or fewer reported	2·1	5·7	1·5	4·8	3·8	3·5**	3·2**
History of chloasma versus no history	0·6	0·6	0·9	0·5	0·4	0·5**	0·6**

* Differs significantly from 1·0, $P<0{\cdot}05$.
** $P<0{\cdot}01$.

adjusted RR being 0·6 and highly significant (95% CI 0·46 to 0·83). Because of this all analyses and reference to 'hair colour' in this text refer to that at 5 years of age, unless otherwise specified.

Each woman was asked whether, if she had no tan in the summer and were to go out in the midday sun for 30 min, her skin would go red and blister or peel later, or whether there would be a milder response. Those reporting that their skin would go red and then blister or peel had a 43% increase in risk compared with those who reported that they would have a milder reaction (RR = 1·4, 95% CI 1·07 to 1·91). After adjusting for hair and skin colour the RR fell to 1·1 and was no longer significant (95% CI 0·81 to 1·52). Women who reported that they would not tan after such an exposure were also more frequent in cases (64·5%) than controls (40·8%), but this too was largely accounted for by the different hair and skin colour of cases and controls. The tendency to freckle after exposure to sunlight was also associated with an increased risk (RR = 1·9) as shown in Table 2. The relationship persisted in each sub-group, except for those with red hair, but the RR fell to 1·4 after adjusting for hair and skin colour and was of borderline significance (95% CI 1·00 to 1·95). Those who reported that they were of Celtic origin had a small and non-significant increase in melanoma risk (RR = 1·3).

The frequency of naevi on the body was assessed by questioning—explaining that, on average, individuals had about twenty moles on their body (Nicholls, 1973)—and by the interviewer observing the skin of the limbs. The reporting of more than average naevi on the

TABLE 3. Relative risk of melanoma associated with a history of various skin diseases

Skin disease	Relative risk
Psoriasis	3·0*
Vitiligo	1·8
Eczema	1·1
Dermatitis	0·6
Acne	0·4*

* Differs significantly from 1·0, $P < 0·05$.

TABLE 4. Site of lesions, by hair and skin colour

	Hair and skin colour				
Site of lesion	Red hair	Blonde hair and fair skin	Blonde hair and dark skin	Brown/black hair and fair skin	Brown/black hair and dark skin
Head and neck	2	2	3	8	7
Arm					
Above elbow	6	18	9	12	4
Below elbow	1	2	3	3	4
Trunk	5	11	8	8	15
Leg					
Above knee	18	21	8	1	7
Below knee	18	24	19	14	15
Total*	50	78	50	46	52
Lesions on head and neck as percentage of total lesions	4%	3%	6%	17%	13%
Lesions below the elbow as percentage of all arm lesions	14%	10%	25%	20%	50%
Lesions below the knee as percentage of all leg lesions	50%	53%	70%	93%	68%

* Data missing for nine cases.

body was associated with a more than three-fold increase in melanoma risk (RR = 3·4). This association persisted for all combinations of hair and skin colour (Table 2) and the adjusted RR remained elevated at 3·2 (95% CI 2·32 to 4·57). The presence of multiple naevi as assessed by the interviewer was also more frequent in cases (8·1%) than controls (2·4%), being associated with a relative risk of 3·7. A history suggestive of chloasma was less frequent in cases than controls (RR = 0·6, Table 2). The reduced risk persisted with all combinations of hair and skin colour, the adjusted RR also being 0·6 (95% CI 0·41 to 0·84).

A history of psoriasis, although rare, was reported significantly more frequently by cases than controls (by 3·2% cases and 1·1% controls, see Table 3. Vilitigo was twice as common in cases than controls (3·2% and 1·8% respectively). There was little difference in the reported history of eczema or dermatitis, but there was a significant deficit of acne reported by the cases (Table 3).

The distribution of the sites of the lesions by hair and skin colour is shown in Table 4. Persons whose complexions made them most resistant to melanoma tended to have the most peripherally distributed lesions. For example, the percentage of lesions on the head and neck increased from 4% in red-haired women to 13% in those with brown or black hair and dark skin (Table 4; χ^2 for trend = 8·6, $P < 0{\cdot}01$); for lesions on the arms, the percentage which was located below the elbow increased from 14% in those with red hair to 50% in those with dark hair and skin (χ^2 for trend = 3·7, $P < 0{\cdot}05$). Similarly for lesions on the legs, the percentage of lesions below the knee increased from 50% to 68% respectively (χ^2 for trend = 6·3, $P < 0{\cdot}01$).

DISCUSSION

This is the largest case-control study of melanoma yet reported and so it has been possible to examine in more detail than was possible in the past the correlation between various risk factors. The study was confined to Caucasian women aged 18 to 54 years old and no information for men or older women is available. Since we performed a number of comparisons between cases and controls, we cannot exclude the possibility that some of the associations are due to chance. In addition, certain information, such as past hair colour and skin diseases, was enquired about retrospectively and may therefore be unreliable. We have no reason to suspect our findings are biassed, however, since they generally agree well with those reported elsewhere.

Red hair colour at age 5 was associated with a tripling of risk and blonde hair with a doubling of risk. Pack *et al.* (1963) had already noted the importance of red hair, although their estimated RR of 10·7 was much higher than we found. In contrast lower RRs of 2·0 and 2·1 respectively can be estimated from the data of Lancaster & Nelson (1957) and Gellin *et al.* (1969). None of these authors specified, however, the age at which this hair colour applied. As mentioned earlier, this is important since its colour may change with age and persons with melanoma are less likely than the controls to report that they had some greying of the hair in adulthood. Why premature greying of the hair should be associated with a protection from melanoma is puzzling. The ages of cases and controls were virtually identical and so cannot explain the difference (Beral *et al.*, 1982). The association is strong and consistent for all natural hair colours. It has not been investigated before and is worthy of further attention. After taking hair colour into account fair skin remains as an independent but weaker risk factor. It is mainly important in those with black or brown hair, where it approximately doubles the risk of melanoma. For those with red or blonde hair it has less effect on risk (Table 2). This might explain why Klepp & Magnus (1979) failed to demonstrate fair skin colour as a risk factor in Norway. Since most Norwegian patients have blonde hair, our findings indicate that skin colour would have little additional effect on the risk of melanoma in that population. Finally, when hair and skin colour are taken into account, eye colour has no additional effect on melanoma risk. Pack *et al.* (1963), Lancaster & Nelson (1957) and Gellin *et al.* (1969) all noted that blue eyes were more frequent in those with melanoma than in controls. While our findings agree with theirs, the association is weak and entirely secondary to the coexistence of blue eyes with red or blonde hair and fair skin.

We have also confirmed that those with melanoma are more likely to burn rather than tan after exposure to sunlight. However, our association is mostly due to the fact that persons with red or blonde hair or fair skin tend to burn in the sun; once this is taken into account the relationship disappears. The same is true, but to a lesser extent, for freckling; although those with melanoma are almost twice as likely to report that they usually develop freckles after exposure to sunlight, the relative risk fell from 1·9 to 1·4 after adjustment was made for skin and hair colour.

Why persons with red hair should have the most centrally distributed lesions, and those with dark hair and skin colour the most peripherally distributed lesions, is curious. In blacks and other dark-skinned ethnic groups most melanomas are found on the sole of the foot (Pack *et al.*, 1963). Might it be that the more resistant people are to melanoma, the more peripherally their lesions are distributed—with blacks at one end of the spectrum and red-haired Caucasians at the other? Before too much speculation however, this observation needs to be examined in another set of data.

One of our most important findings is the more than three-fold increase in melanoma risk associated with the reporting of above-average numbers of naevi on the body. This strong relationship persisted for all hair and skin colours. Although this may be, at least in part, because patients with melanoma tend to over-report the number of naevi they have, there was close aggreement between the RRs associated with the self-reporting of above average numbers of naevi (RR = 3·4) and with interviewers' assessments that the individuals had multiple naevi on their limbs (RR = 3·7). Milton (1977) found that two-thirds of patients reported that their melanoma arose in a pre-existent mole and in one-third of cases there was histological evidence of that (McGovern, 1976). Certain types of naevi, especially junctional naevi and the B-K type are particularly liable to become malignant (McGovern, 1976; Reimer *et al.*, 1978). If a large proportion of melanomas do arise in pre-existent naevi, it is not surprising that individuals with large numbers of them are especially prone to melanoma. This may also be relevant for the unusual anatomical distribution of melanoma. Since the majority of naevi are on the trunk (Nicholls, 1973) this might explain why a disproportionate number of melanomas arise there. Furthermore, junctional naevi are especially common on the trunk (Rippley & Whiting, 1977). Unfortunately we did not enquire about the sites of the naevi nor were individuals examined to establish how many lesions were of the junctional or B-K type. This should be done in the future.

The increased risk of melanoma in those with a history of vitiligo has also not been reported before, although analogous associations have long been recognized in animals, especially horses (Hadwen, 1931). By contrast, a history of hyperpigmentation (chloasma) rather than de-pigmentation appears to protect against melanoma. Adams *et al.* (1981) also reported that a smaller proportion of cases than controls gave a history suggestive of chloasma. A history of acne also appears to be associated with a lowered risk of melanoma, regardless of hair colour. The increased production of sebum by persons with acne may account for this, since skin surface lipids significantly reduce transmission of ultraviolet radiation (Beadle & Burton, 1981). Finally, considering the controversy surrounding the use of photochemotherapy for psoriasis, the significantly higher frequency of psoriasis in cases than controls needs to be investigated further. Unfortunately we did not ask about the type of treatment which individuals had received.

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Clonal heterogeneity in curetted human epidermal cancers and precancers analysed by flow cytometry and compared with histology

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SUMMARY

DNA frequency distributions analysed by single nuclei flow cytometry were studied in sixty-five curetted human epidermal tumours, i.e. five actinic keratoses (AK), seven Bowen's diseases (BO), nine squamous cell carcinomas (SCC), forty-three basal cell carcinomas (BCC) and one baso-squamous carcinoma (BSC). Seventy-five per cent (16/21) of the samples with squamous cell differentiation (AK, BO and SCC) showed features suggestive of more than one stem cell population, against 24% of the pure BCC samples (11/43).

The DNA indices for the tumours, i.e. the ratio between the DNA content of the tumour stem cell line G_1 cells and normal epidermis G_1 cells were calculated. For BCC and SCC a preponderance was found for near-diploid and near-tetraploid cell clones. The precancerous lesions contained clones with more broadly scattered DNA indices.

The fractions of cells in S and G_2M and $S + G_2M$ phases were calculated for the samples with only one detectable stem cell population. For the squamous cell tumours and the nodular (but not the superficial) BCC, these fractions were significantly different from the unaffected skin of patients with multiple epidermal cancers. The usefulness of cell cycle fraction determinations for curetted tumours is discussed.

The cellular DNA content in human epidermal tumours has been studied by photometry for many years. Initially the photometric measurements were performed on tissue sections, as a rule Feulgen-stained (Leuchtenberger, Leuchtenberger & Davis, 1954; Atkin & Richards, 1956; Kint, 1963; Manocha, 1969; Manocha, Steele & Stich, 1969; Ehlers, 1968; Ehlers & Stephan, 1972; Ehlers & Herbstreit, 1973). This technique has the advantage of simultaneous microscopic inspection of the cell measured, but it is laborious and time consuming, and the

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results may be inaccurate due to the influence of sectioned nuclei. In 1971 Schumann *et al.* reported photometry used on suspensions or single nuclei prepared from human epidermal tumours. Since then DNA analyses by flow cytometry have been further refined.

Flow cytometric DNA analysis yields information on ploidy and the distribution of cells in the various phases of the cell cycle according to the nuclear DNA content. For normal human epidermis we have previously studied the methodological variation and influences on the cell cycle fractions of age, sex, localization, time of day and biopsy trauma (Frentz, Møller & Christensen, 1980; Frentz, Møller & Keiding, 1982; Frentz & Møller, 1983).

The present study represents our initial approach on extending the use of the method to human epidermal tissue with malignant or premalignant features. The immediate purpose was to verify whether the DNA frequency distributions in these tissues differed basically from normal human epidermis, and to estimate if significant differences might be expected between histologically different tumours.

METHODS

We studied sixty-five human epidermal malignant or premalignant tumours obtained from forty-eight dermatological out-patients (nineteen females aged 49–89 yrs, mean 73; twenty-nine males aged 45–91 yrs, mean 65). Thirty-five subjects had a history of multiple epidermal malignancies.

The tumours were generally rather small and superficial, measuring 0·5–3 cm in diameter. Local anaesthesia was injected deeply intradermally (mepivacaine or carbocaine 2% without adrenaline). The total amount of tissue immediately removable by curettage without violent scraping was sampled. The tissue samples were divided in two; one half was processed for routine histological examination and the other was prepared for flow cytometry.

The histological classification of the tumours followed conventional criteria. The solid type of basal cell carcinomas was divided into a superficial type and a nodular type on clinical and histological features.

The preparation of tissue for flow cytometry followed the procedures previously described (Møller & Larsen, 1979; Frentz, Møller & Larsen, unpublished). The DNA content of individual nuclei was measured according to our standard procedure (Frentz *et al.*, 1980) in a flow cytometer combined with a multichannel height-analyser (cytofluorograph Bio Physics System Inc., type 4802A and 6300A). The G_1 peak of normal epidermis was placed in channel number 37 (total channel number 190). The instrument settings remained stationary during the procedure each day. The stability of the fluorescence signals was checked by running standard samples containing polysterene particles, normal human epidermal nuclei or trout erythrocytes before and after the tumour samples. Every third or fifth run contained one of the standard samples alone.

For each sample we evaluated the number of clones detectable and the DNA index, i.e. the ratio between the G_1 peak DNA content of the cell line(s) in the sample and the G_1 peak DNA content of the normal epidermal cells. The fractions of cells in G_1, S and G_2M phases were calculated for the histograms with features suggestive of a monoclonal cell population according to the previously described method (Frentz *et al.*, 1980). Unaffected epidermis from patients with multiple epidermal cancers was used as control (Frentz & Møller, 1982).

Pluriclonality was considered present when more than one 'G_1 peak' was seen in the histogram (Fig. 1a), when the coefficient of variation for the G_1 peak was 11·5 or higher (Fig. 2)

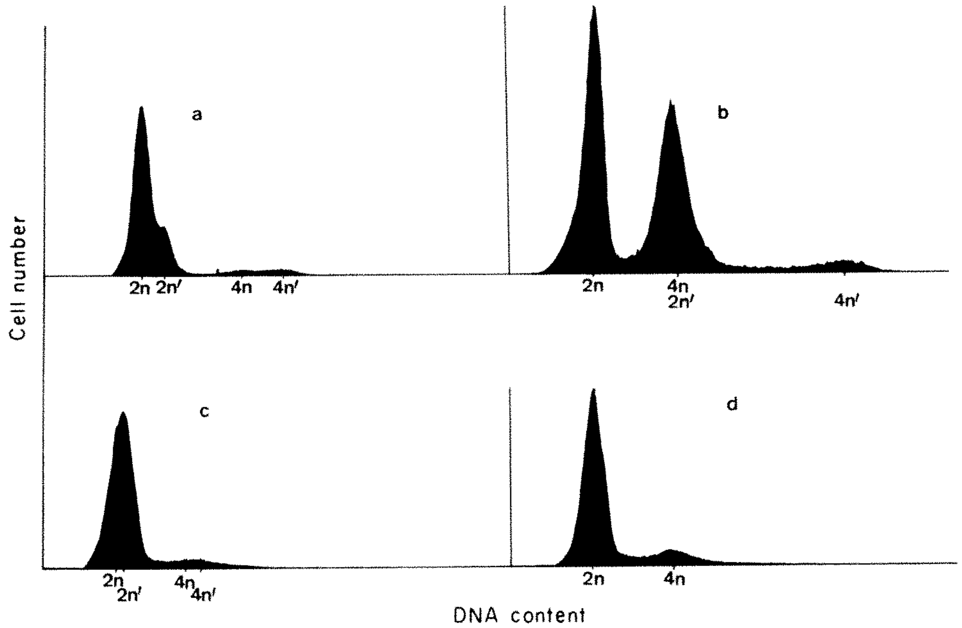

FIGURE 1. DNA frequency distributions obtained from different malignant epidermal tumours. The abscissa indicates the relative fluorescence intensity parallelling the DNA content, the ordinate shows the relative number of cells. (a) Basal cell carcinoma. Two different 'G_1 peaks' are immediately recognized, indicating at least one aneuploid clone of cells corresponding to the calculation procedure for the statistical analysis, which separates peaks when the peak DNA content differed by approximately twice the mean value of the CVs for the peaks. (The DNA indices for the clones are calculated to be 1·0 and 1·3.) (b) Squamous cell carcinoma. A high 'G_2M' peak (G_2M fraction $\geq 20\%$) indicates an additional near tetraploid tumour clone, the 'G_2M' peak of which is found at the octaploid level (DNA indices: 1·0 and 2·0). (c) Basal cell carcinoma. The CV of the G_1 peak is $> 11{\cdot}5$ and the G_1 peak is obviously skewed, indicating the presence of two clones with slightly differing DNA content (see Fig. 2). (DNA indices: 1·0 and 1·1.) (d) Basal cell carcinoma. The CV of the G_1 peak is $> 11{\cdot}5$. No obvious skewness in the G_1 curve is seen. The DNA indices of two presumably different DNA stem cell lines are technically indistinguishable (see Fig. 2 and legend).

and when the G_2M fraction reached 20% or a higher value with a simultaneous occurrence of a peak at the octaploid level (Fig. 1).

The DNA index was calculated by means of the following equation (Vindeløv *et al.*, 1980):

$$\text{DNA index (sample)} = \frac{\text{channel number } G_1 \text{ peak (sample)}}{\text{channel number } G_1 \text{ peak (standard)}} \times k,$$

where k represents the ratio between the G_1 peak DNA content of the standard sample in question and the normal epidermal nuclei.

In histograms with features suggestive of pluriclonality a DNA index was calculated for each clone identifiable. The principles used for determination of the G_1 peaks for the individual clone are illustrated and commented on in Fig. 1. The limits for euploidy were set at $0{\cdot}9 \leq$ DNA index $\leq 1{\cdot}1$, the CV of DNA index determination by direct calculation for normal epidermis being 5% (Frentz, Møller & Larsen, unpublished). Thus aneuploidy was present beyond these

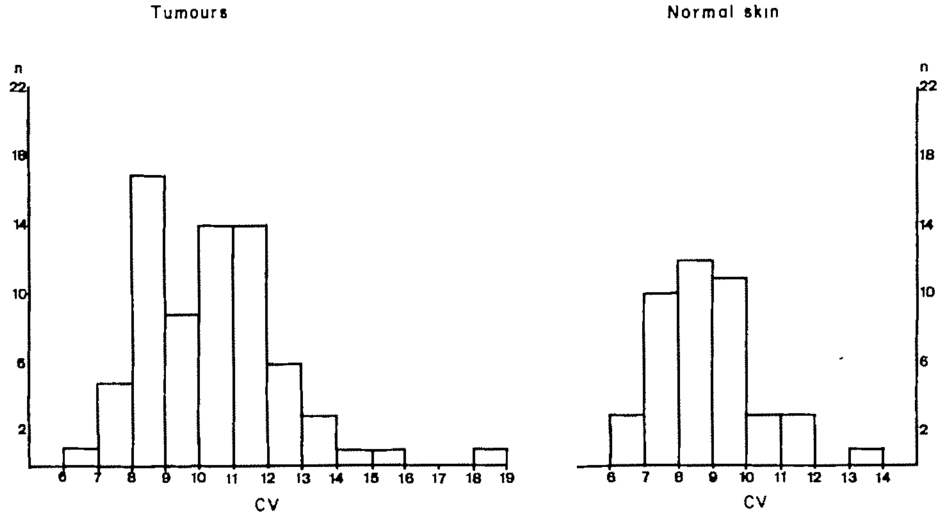

FIGURE 2. The distribution of the CVs for the G_1 peaks obtained from the epidermal tumours and from the unaffected skin from patients with multiple epidermal tumours (Frentz & Møller, unpublished). The distribution of the tumour sample CVs has a second peak around 11, the first peak appearing at CV 8–9 in accordance with the single peak for the unaffected skin. Based on this we classified samples exhibiting CVs > 11·5 as pluriclonal, while samples with CVs ≤ 11·5 were classified as monoclonal.

TABLE 1. The number of clones detected in sixty-five curetted tumours. The pluriclonal samples are listed according to the criteria used for identification of the additional clone(s)

				Criteria for pluriclonality		
Histology	Total no.	Monoclonal samples	Pluriclonal samples	I one G_1 peak	II CV > 11·5	III $G_2M \geq 20$
Basosquamous carcinoma	1	0	1	0	1	0
Nodular solid type BCC	23	18	5	1	3	1
Superficial solid type BCC	18	12	6	2	4	0
Adenoid type BCC	2	2	0	0	0	0
Group of pure BCC	(43)	(32)	(11)			
Actinic keratosis	5	2	3	3	0	0
Bowen's disease	7	1	6*	1	4	2
Group of precancers	(12)	(3)	(9)			
Squamous cell carcinoma	9	2	7	1	4	2
Group of squamous cell tumours	(21)	(5)	(16)			
TOTAL	65	37 (57%)	28 (43%)	8	16†	5

* One of these tumours fulfilled both criterion II and III.

† Half of the G_1 peaks were asymmetrical with features suggestive of two clones with differing DNA indices.

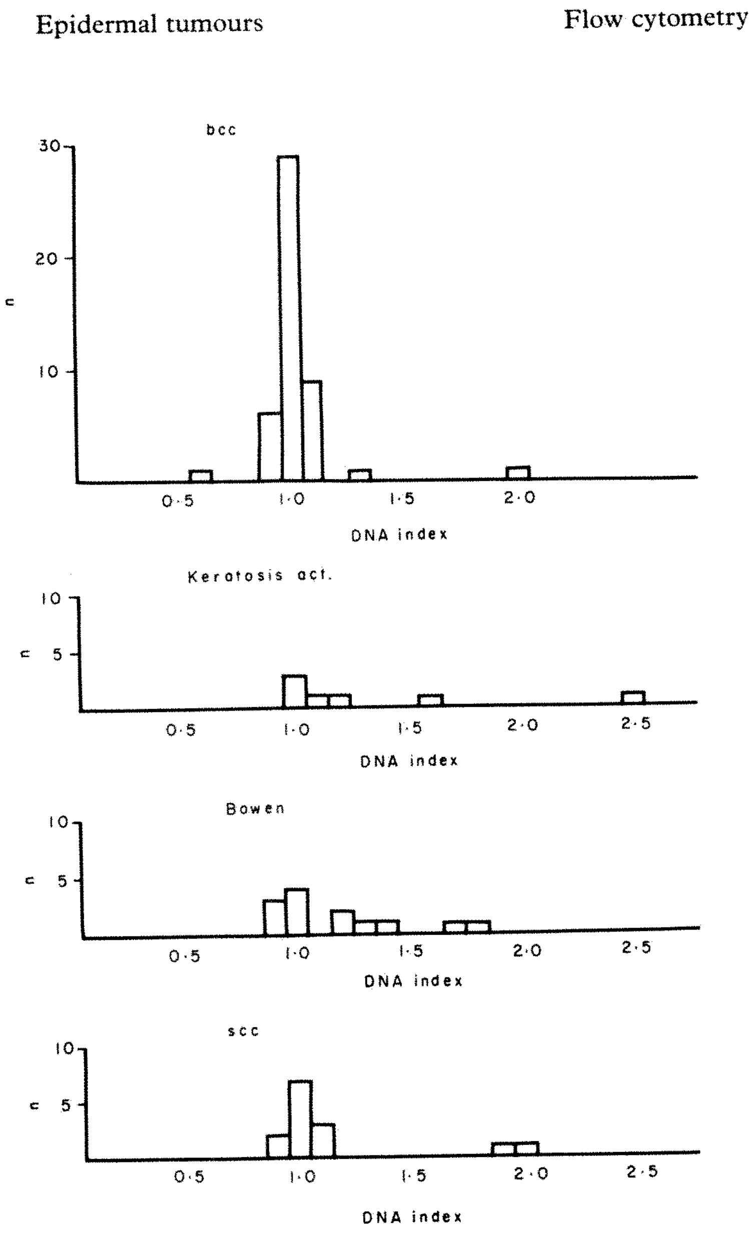

FIGURE 3. DNA index distributions for the different tumour groups investigated. Preponderance is seen for near-diploid DA lines in BCC, for near-diploid and near-tetraploid DNA stem cell lines in SCC and more scattered DNA stem cell lines in the preinvasive lesions.

limits and additionally by definition also in the samples which contained more than one stem cell line.

RESULTS

Twenty-eight of the sixty-five tumours (43%) were classified as pluriclonal according to the criteria used. One of the BO samples fulfilled two criteria for pluriclonality. Table 1 relates the frequency of pluriclonality to the histological group. Seventy-five per cent of the twelve precancerous lesions were pluriclonal. This equalled the percentage for the invasive SCC. In contrast only 24% of the pure BCC were characterized by this feature. The difference in the frequency of pluriclonality between BCC and SCC was significant ($P = 0{\cdot}02$, Fischer's exact test), and between the BCC and the entire group of squamous cell tumours it was highly significant (chi square 2K contingency test, $P = 0{\cdot}00012$). Neither the patients' age, sex nor body site influenced the frequency of pluriclonality significantly, each parameter tested isolated (plots, Fischer's exact test, Fischer's exact test performed for squamous cell tumours located on face and scalp against extremities, respectively). Pluriclonality was not more frequent in tumours from patients with multiple lesions than in the remainder (chi square 2K contingency test, $P = 0{\cdot}27$).

DNA indices were calculated for fifty-six tumours containing eighty-three clones (the remaining nine tumours escaped evaluation due to a technical error). The DNA index determination did not reveal aneuploid clones in the monoclonal tumours and thus in this study aneuploidy equalled pluriclonality. The DNA indices calculated for the pure BCC, the AK, the BO and the SCC are graphically summarized in Fig. 3; the single BCC with two stem cell lines (DNA indices 1·0 and 1·1) is omitted.

The fractions of cells in S, G_2M and $S + G_2M$ phases calculated for each of the monoclonal tumours are summarized in Table 2. Within each group of the tumours a wide range occurred. However, the group of squamous cell tumours and the nodular type of BCC could statistically be separated by the values from the unaffected epidermis of multiple skin cancer patients (for SC tumours; $P < 0{\cdot}01$ for G_2M and $S + G_2M$; $P < 0{\cdot}05$ for S; for nodular BCC: $P < 0{\cdot}01$ for $S + G_2M$; $P < 0{\cdot}02$ for S and $P < 0{\cdot}05$ for G_2M, unpaired two-tailed t test). In contrast, the

TABLE 2. The S, G_2M fractions and the $S + G_2M$ fraction for the monoclonal tumours

		S		G_2M		$S + G_2M$	
Tumour type	n	$\bar{x} \pm$ s d.	(range)	$\bar{x} \pm$ s.d.	(range)	$\bar{x} \pm$ s.d.	(range)
Nodular solid BCC	18	7·1 ± 2·1	(4·4–11·9)	5·3 ± 4·0	(1·5–19·9)	12·4 ± 4·8	(6·8–26·9)
Superficial solid BCC	12	5·7 ± 2·2	(3·0–10·7)	3·3 ± 1·5	(1·3–6·2)	9·0 ± 2·8	(6·0–14·4)
Adenoid BCC	2	9·0	(5·3–12·7)	5·0	(2·9–7·1)	14·0	(8·2–19·8)
Actinic keratosis	2	8·9	(5·9–11·9)	6·5	(4·3–8·6)	15·4	(10·2–20·5)
Bowen's disease	1	7·7		8·1		15·8	
Squamous carcinoma	2	8·2	(6·0–10·4)	6·8	(6·7–6·9)	15·0	(12·7–7·4)
Group of squamous cell tumours	5	8·4 ± 2·7		6·9 ± 1·7		15·3 ± 4·0	
Clinically normal skin of patients with multiple carcinomas	19	4·7 ± 3·3		3·0 ± 2·2		7·7 ± 5·2	

values for the superficial type of BCC were not statistically different from the controls ($P > 0{\cdot}2$ for all three measurements).

When the values were compared for the different histological groups the superficial BCC differed from the nodular BCC by having a smaller $S + G_2M$ fraction ($P < 0{\cdot}05$). No significant differences were found between the nodular BCC and the group of squamous cell tumours. Age, sex and body site analysed separately did not influence the values of the eighteen nodular BCC significantly (linear regression analysis, t tests). The five nodular BCC which were induced by actinic damage did not differ significantly in the size of the cell cycle fractions from the remaining thirteen. The nodular BCC from patients developing multiple skin cancers, however, differed from those from patients with solitary lesions by having a higher S fraction ($P < 0{\cdot}05$, t test).

DISCUSSION

In the present study on sixty-five human epidermal cancers and precancers, ninety-four stem cell lines or clones were identified. This is a minimum figure because considerable limitations in the power of resolution are imposed by the method.Thus it is impossible to recognize minute clones and to distinguish clones with only slightly differing DNA content. The limit between samples assessed to contain one clone and two adjacent clones was settled to 11·5 because the tumour samples obviously had a second CV peak for the G_1 fraction at that level when compared with unaffected epidermis (Fig. 2). If a CV value of 11 or 12 had been chosen instead the number of pluriclonal samples would increase and decrease respectively by 5.

Twenty-nine extra clones in twenty-eight pluriclonal tumours give a minimum of twenty-nine aneuploid clones. One of the clones identified in each tumour might contain the normal cells. However, in a few tumours the histological examination suggested that the proportion of normal cells was too small for independent recognition of a normal cell clone in the DNA distribution. No obvious correlation was found between the number of normal cells in the histological sections and the various values obtained by flow cytometry. However, this approach was considered too inaccurate for final conclusions. On the other hand at least one clone with an euploid DNA content was found in each of the tumours. When the DNA index for each clone was used exclusively for evaluation of aneuploidy only fourteen clones were recognized as aneuploid, regardless of distinct pluriclonality in several of the remaining samples. Thus the indirect DNA index determination by means of non-intrinsic standards in the present study is useful for broad estimations of ploidy only.

In the tumours here investigated pluriclonality equalled aneuploidy. Using the criteria outlined approximately 43% of the total number of tumours were evaluated as aneuploid (78% of the SCC and 24% of the BCC). The detailed figures are in good agreement with those reported by Schumann *et al.* (1979) (80% and 23% respectively). It is remarkable that aneuploidy occurred in preinvasive tumours with the same frequency as in invasive SCC. Thus the presence of aneuploidy cannot by itself be regarded as a sign of clinical aggression. The absence of correlation between aneuploidy and prognosis has also been stated for malignant tumours appearing in other organ systems (Jakobsen, Bichel & Sell, 1979; Dixon & Carter, 1980; Thommesen *et al.*, 1980). Instead aneuploidy tends to correlate with the cellular and nuclear pleomorphism as evaluated by histological examination (Jakobsen, 1979). In contrast to the findings of Barlogie *et al.* (1980) for a variety of human solid tumours, neither age nor sex correlated with aneuploidy in the present material.

The DNA indices in the present study ranged from 0·6–2·5 indicating an evident clonal heterogeneity. For the distribution of the DNA indices within and between the different groups of tumours (Fig. 3) trends were noted for predominantly near-diploidy for the BCC, near-diploidy and near-tetraploidy for the SCC and more scattered DNA index values for the preinvasive tumours. However, extension of the measurements beyond the double value of the octaploid level might reveal additional clones. Using cytophotometry on Feulgen-stained tissue sections, Ehlers & Stephan (1972) and Manocha *et al.* (1969) found broadly scattered DNA frequency distributions for separate human precancerous skin lesions suggesting a multiclonal origin.

For tumour tissue sampled by curettage as outlined various amounts of normal epidermis will be present in almost all samples as also seen in the histological sections. Thus, even in apparently monoclonal DNA distributions both normal and neoplastic tissue might contribute to the calculated cell cycle fractions. This interferes with the recognition of correlations between the cell cycle fractions and the various clinical and histological data, and may play a role in the overlapping values for the unaffected epidermis and the group of superficial BCC. On the other hand, the two subgroups of solid BCC could statistically be distinguished from each other on the $S+G_2M$ fraction, which was higher for the nodular type. Weinstein & Frost (1970) found higher ^{3}HTdR labelling indices in the superficial type than in the nodular type and considered this difference was due to methodological factors. The same may be true for our findings, because the curetted superficial BCC samples as a whole in the histological sections contained a larger amount of apparently normal epidermis than did the nodular BCC. For these various methodological problems it was not considered worthwhile performing multivariate analyses for the cell cycle fractions calculated.

For the nodular BCC, significantly higher S fractions were found for patients with multiple cancers than for those with only a single tumour. This might parallel our finding of a higher S fraction in the perilesional/carcinogenically exposed skin of multiple epidermal carcinoma patients compared with control samples (Frentz & Møller, unpublished). This question deserves further study.

Curettage is a simple and convenient technique for removing small and superficial epidermal tumours . The present study indicates that DNA measurements by flow cytometry can provide information on clonal heterogeneity and ploidy when used for curetted tumour samples. The value of calculating the fractions of cells in the different cell cycle phases from curetted material is uncertain at present. However, flow cytometry is a useful tool, which is currently undergoing rapid development.

ACKNOWLEDGMENTS

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Macrophage–T lymphocyte relationships in man's contact allergic reactions

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SUMMARY

Seventeen biopsies from contact allergic reaction sites in sensitized patients were studied by electron microscopy and immunocytochemistry. Very intimate appositions of macrophages to T lymphocytes were frequently observed in the dermis at 6, 8 and 12 hours after application of the allergen. Such juxtapositions were seen less frequently after 24, 48 and 72 hours. These appositions seem to indicate that macrophages play an antigen-presenting role in human contact allergic reactions, as Langerhans cells seem to do.

The interactions occurring among lymphocytes, Langerhans cells and macrophages in contact allergic dermatitis have previously been investigated. Although appositions between macrophages and lymphocytes were detected many years ago in contact allergic reactions in man (Wolff & Braun-Falco, 1971) and in guinea-pig (Medenica & Rostenberg, 1971), a functional role for macrophages in the pathogenesis of contact allergic dermatitis has not been defined. On the other hand, the close apposition of lymphocytes to Langerhans cells (Silberberg, 1973; Silberberg, Baer & Rosenthal, 1974; Silberberg, Baer & Rosenthal, 1976; Silberberg-Sinakin & Thorbecke, 1980) has been interpreted as evidence of an important role of Langerhans cells in eliciting cutaneous sensitization.

The aim of the present study was to provide a better definition of the interactions between macrophages and T lymphocytes in contact allergic reactions to simple chemicals in man.

METHODS

For electron microscopy, fifteen biopsies were taken from patch test areas in six patients with contact allergic dermatitis, 3, 6, 8, 12, 24, 48 and 72 h after application of allergens (0·5%

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TABLE 1. Materials and methods

Time of biopsy (hours)	Patients	Allergen applied	Fixing medium for electron microscopy	Immunocytochemistry (frozen unfixed sections)
3	S.R.	Co	K	done
	A.B.	Ni	not done	done
6	M.C.	Cr	K	done
	S.R.	Co	K	done
8	T.A.	Cr	GC	not done
12	T.A.	Cr	GC	not done
	N.U.	Cr	GC	not done
	M.C.	Cr	K	done
	A.B.	Ni	not done	done
24	N.U.	Cr	GC	not done
	M.C.	Cr	K	done
	S.R.	Co	K	done
48	T.A.	Cr	GC	not done
	M.C.	Cr	K	done
	O.M.	Ni	K	done
	A.B.	Ni	K	done
72	M.C.	Cr	K	done

Co: 3% cobalt sulphate, in petrolatum
Ni: 3% nickel sulphate, in petrolatum
Cr: 0·5% potassium bichromate, in petrolatum
K: Karnovsky's fluid
GC: 4% glutaraldehyde, in cacodylate buffer

potassium bichromate, 3% nickel sulphate, 3% cobalt sulphate, in petrolatum). Some specimens were fixed in Karnovsky's fluid, postfixed in osmium tetroxide, dehydrated in acetone, embedded in araldite; the other specimens were fixed in 4% glutaraldehyde in cacodylate buffer, postfixed in osmium tetroxide, dehydrated in acetone and embedded in Epon (Table 1). Ultrathin sections were obtained by means of a LKB ultramicrotome and stained with uranyl acetate and lead citrate.

For immunocytochemical procedures, twelve biopsies were taken from four patients (Table 1). On 4-μm frozen sections from unfixed specimens, double step techniques were performed, using OKT3 (Ortho) and Leu1 (Becton Dickinson) monoclonal antibodies, to identify T cells. The endogenous peroxidases were previously blocked according to Streefkerk (1972). Then the sections were incubated with the monoclonal antibodies diluted 1:10 in PBS, at room temperature for 1 h; after washing in PBS for 1 h, the sections were incubated with a peroxidase conjugate F(ab')2 fragment goat anti-mouse IgG (Cappel) for 1 h. The peroxidase activity was revealed according to the method of Graham & Karnovsky (1966). As controls, a purified mouse IgG fraction (Pel-Freeze) and the incubation medium without monoclonal antibodies were used. Further details of the immunocytochemical procedures we used are extensively reported elsewhere (De Panfilis *et al.*, 1979).

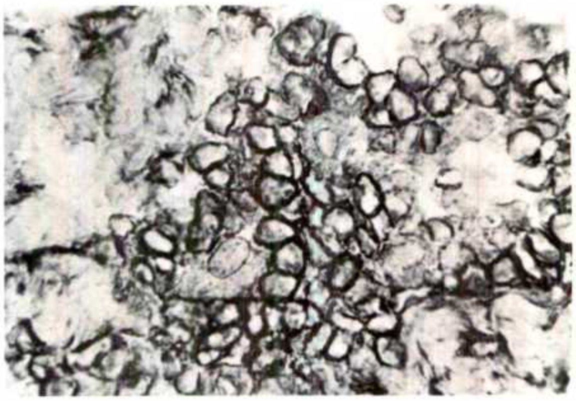

FIGURE 1. Contact allergic reaction, at 12 h after application of 3% nickel sulphate. Among OKT3-positive small mononuclear cells (T lymphocytes), large OKT3-negative cells (possibly macrophages) are seen. Double layer immunocytochemical technique (× 600).

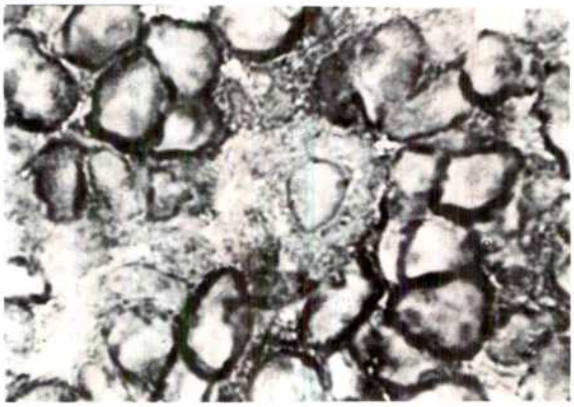

FIGURE 2. Contact allergic reaction at 12 h after application of 3% nickel sulphate. A detailed view of one of the OKT3-negative cells of the Fig. 1; cytoplasmic processes are more clearly seen. Double layer immunocytochemical technique (× 1500).

RESULTS

Three hours after application of the allergens, evident signs of cellular infiltration were not seen. At 6, 8, and 12 h, infiltrating cells (mainly macrophages and lymphocytes) were present in the dermis.

Immunocytochemical findings demonstrated the frequent occurrence of relationships between OKT3- or Leu1-positive T lymphocytes and unlabelled cells which, due to their large size, irregular cytoplasmic outlines and clear nucleus, may be regarded as macrophages (Figs 1 and 2).

In ultrathin sections lymphocytes and macrophages appeared in intimate apposition: the cell membranes of lymphocytes and macrophages ran parallel for large distances with a gap not exceeding 200 Å (Figs 3 and 4). Sometimes cytoplasmic processes of macrophages enveloped

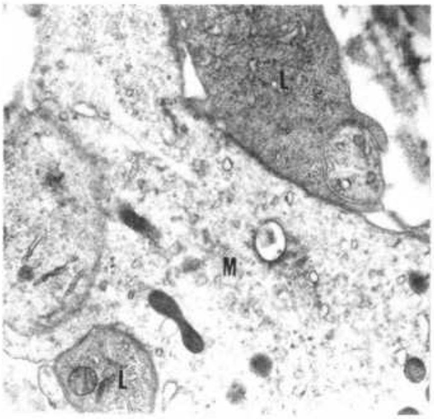

FIGURE 3. Contact allergic reaction, at 12 h after application of 0·5% potassium bichromate. Very close juxtapositions between lymphocytes (L) and a macrophage (M) (× 8640).

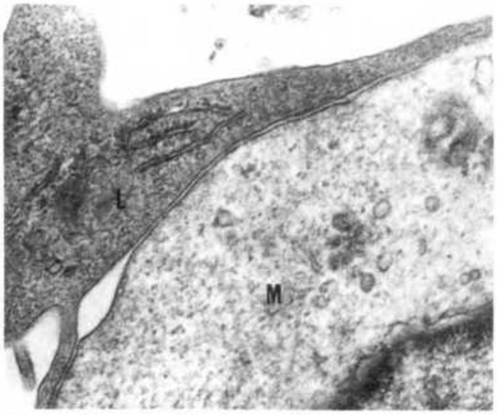

FIGURE 4. Contact allergic reaction, at 12 h after application of 0·5% potassium bichromate. Very extended contact area between a lymphocte (L) and a macrophage (M); the gap between the cell membranes does not exceed 200 Å (× 23 200).

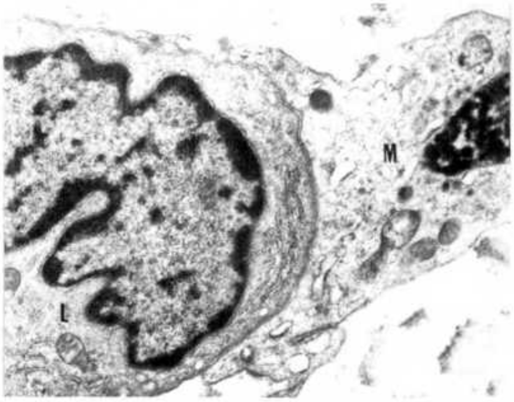

FIGURE 5. Contact allergic reaction, at 12 h after application of 0·5% potassium bichromate. A large cytoplasmic process of a macrophage (M) envelops a lymphocyte (L) (× 8640).

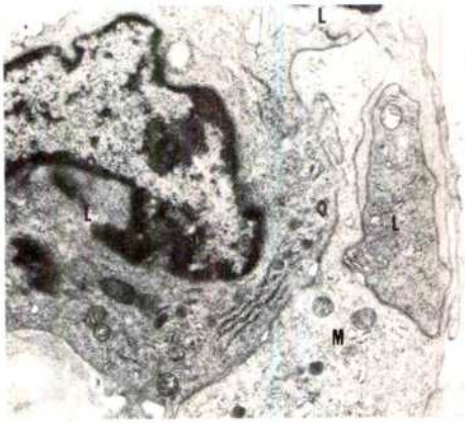

FIGURE 6. Contact allergic reaction, at 12 h after application of 0·5% potassium bichromate. An elongated process of a macrophage (M) is in intimate contact with surrounding lymphocytes (L) (× 6800).

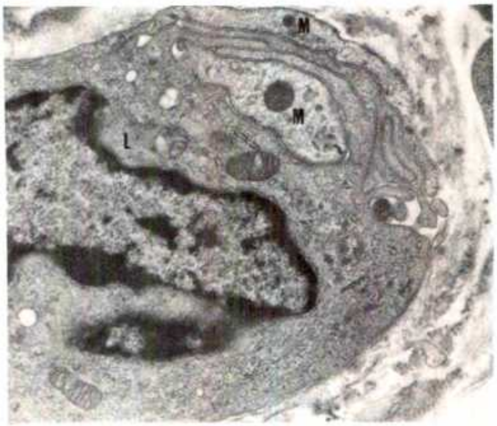

FIGURE 7. Contact allergic reaction, at 12 h after application of 0·5% potassium bichromate. A cytoplasmic process of macrophage (M) penetrates a lymphocyte (L) (× 8860).

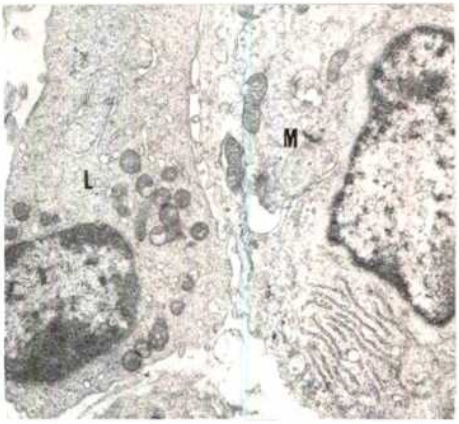

FIGURE 8. Contact allergic reaction, at 48 h after application of 0·5% potassium bichromate. A lymphocyte (L) lies near a macrophage (M): only minimal contact areas are seen (× 6640).

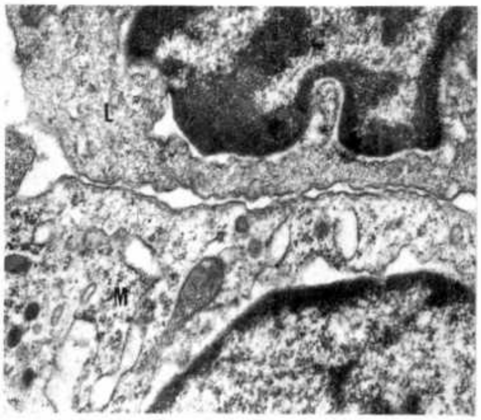

FIGURE 9. Contact allergic reaction, at 48 h after application of 0·5% potassium bichromate. The cell membranes of a lymphocyte (L) and of a macrophage (M) are not intimately apposed and do not run parallel (× 16,200).

lymphocytes (Fig. 5); not infrequently such cytoplasmic processes appeared enclosed in invaginations of the peripheral cytoplasm of the lymphocytes (Figs 6 and 7).

At 24, 48 and 72 hours both the lymphocytes and the macrophages became more numerous. However, macrophages and lymphocytes were less frequently juxtaposed than in earlier stages. When macrophages and lymphocytes were near each other, their cell membranes were not so intimately apposed as at earlier times (Fig. 8) and did not run so straight and parallel, but showed irregular profiles (Fig. 9).

Cells with Birbeck's granules were never found in the dermis. In the epidermis, where scattered lymphocytes occurred, only once (at 12 h) was a close apposition between lymphocytes and an undamaged Langerhans cell found (Fig. 10).

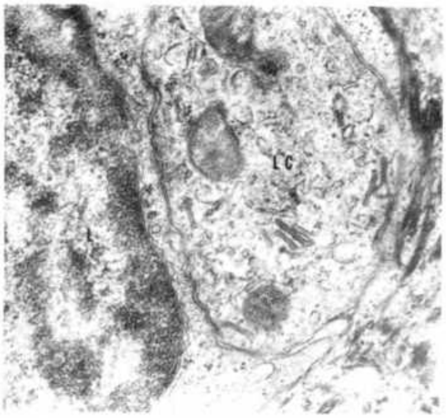

FIGURE 10. Contact allergic reaction, at 12 h after application of 0·5% potassium bichromate. Juxtaposition of a lymphocyte (L) to a Langerhans cell (LC) in the epidermis, (× 20,500).

DISCUSSION

In vivo and *in vitro* investigations suggest that Langerhans cells play an antigen-presenting role in the afferent limb of the sensitization process (Stingl, Tamaki & Katz, 1980; Silberberg-Sinakin & Thorbecke, 1980). The possibility that Langerhans cells may play the same role in the efferent limb was suggested only on the basis of the findings, in sensitized man and guinea-pigs, of close apposition between lymphocytes and Langerhans cells in contact allergic reaction sites (Silberberg, Baer & Rosenthal, 1974; Silberberg, Baer & Rosenthal, 1976). In our specimens we found only one Langerhans cell juxtaposed to lymphocytes in the epidermis. Contacts between Langerhans cells and lymphocytes appear not to be a constant finding of contact allergic reactions in man.

By contrast, after 6 h or more, intimate appositions were frequent in the dermis between macrophages and lymphocytes, thus confirming the findings of other workers in man and guinea-pig (Wolff & Braun-Falco, 1971; Medenica & Rostenberg, 1971). The results of our study with monoclonal antibodies demonstrate that in contact allergic reactions in man the lymphocytes infiltrating the dermis and juxtaposed to macrophages are T lymphocytes. Furthermore, *in vitro* investigations demonstrated that the interaction between macrophages and T lymphocytes requires a physical contact of such cells (Beller, Farr & Unanue, 1978). Therefore, we believe that macrophages, whose antigen-presenting function to T lymphocytes is now widely accepted, may play an important role in the efferent limb of allergic contact sensitivity to simple chemicals in man.

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Enhanced comedo formation in rabbit ear skin by squalene and oleic acid peroxides

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SUMMARY

The comedogenicity of UVA-irradiated and non-irradiated substances (squalene, oleic acid, tetradecane, isopropyl myristate, squalane and liquid paraffin) was evaluated by surface microscopy and histological examination after treating the ventral skin of rabbit ears with these substances. The lipid peroxide levels of these substances were also measured. Squalene itself was scarcely comedogenic but squalene peroxides were highly comedogenic. Both oleic acid and its peroxides were able to induce fairly large comedones and there was a good correlation between the lipid peroxide levels and the size of the comedones. The specimens biopsied from these comedones showed marked hyperplasia and hyperkeratosis of the epithelium in the follicular infundibulum and marked proliferation of the sebaceous glands.

Although free fatty acids might play a role in the pathogenesis of acne, it is proposed that squalene and free fatty acids in sebum may be less comedogenic than their peroxides.

Squalene, one of the components of human sebum, has been shown to be as comedogenic as free fatty acids in the rabbit ear model (Kligman, Wheatley & Mills, 1970) and there is a high squalene content in the lipids of the larger sebaceous glands of acne patients (Summerly *et al.*, 1976). Wheatley (1979) stated that, if comedogenesis is due to a lipid component of sebum, the most likely candidate is squalene.

On the other hand, squalene produces its peroxides in large amounts in skin surface lipids and these peroxides cause a strong irritation on stripped human skin, and a growth inhibition in cultured human keratinocytes (Yamamoto *et al.*, 1982).

The present study was undertaken to examine the comedogenicity of squalene, oleic acid and their peroxides on the rabbit ear model using surface microscopic and histological techniques and to elucidate the role of their peroxides in the course of comedogenesis.

METHODS

Squalene (purity = 97%) and oleic acid (purity = 99%) were purchased from Eastman Kodak

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Co., Rochester, N.Y. and P-L Biochemicals, Inc., Milwaukee, Wis., respectively. Isopropyl myristate (Nakarai Chemicals, purity = 95%), n-tetradecane (Kanto Chemicals, purity = 99·5%), squalene (Wako Pure Chemicals, purity = 99·4%) and liquid paraffin (Nakarai Chemicals, $n_D^{20} = 1{\cdot}4673$) were used as controls. These chemicals were used without further purification.

Two ml of each substance was poured into a glass schale (diameter = 5·5 cm) and received 18 h of UV-A irradiation from a 400 W fluorescent sunlamp (Toshiba H400PL lamp, peak emission = 365 nm). The approximate dose of UV-A was $1{\cdot}2 \times 10^{-3}$ joule/sec/cm^2. After UV-A irradiation, each substance was kept in a light-intercepting ampoule at 5°C.

Lipid peroxide levels were fluorescent-spectrophotometrically determined according to the TBA method (Yagi, 1976) and expressed in terms of the malondialdehyde value (MDA nmol/ml).

The procedures for evaluating the comedogenicity of chemicals on the ventral surface of rabbit ears have been fully described by Motoyoshi (1983). Twelve young male Japanese conventional white rabbits and twelve New Zealand White rabbits were used for the experiment. The experiment was repeated in duplicate on both species.

A UVA-irradiated substance was smeared on to the ventral surface of the ears of two rabbits. The same non-irradiated one was smeared on to the opposite ears. Both substances were applied daily for 2 weeks.

The diameters of ten follicular eruptions per ear were measured daily under a Leitz MZ microscope. The average diameters of twenty follicular eruptions from two rabbits were obtained and compared with each other.

Since squalene and oleic acid had a tendency to produce their lipid peroxides in large amounts by UVA-irradiation, it was determined whether the lipid peroxide levels of squalene and oleic acid peroxides were proportional to the degree of comedo formation on the rabbit ear model. Nine, 18 and 36 h UVA-irradiated squalene and oleic acid were evaluated for their comedogenicity on the ears of New Zealand White rabbits. Two rabbits were used for the experiment for each substance. Specimens biopsied after 2 weeks' treatment were fixed in 10% formalin fluid, processed and embedded in paraffin blocks. Vertical sections were cut and stained with haematoxylin and eosin.

RESULTS

Lipid peroxide levels

The lipid peroxide levels of six substances with or without UVA-irradiation are summarized in Fig. 1. Five non-irradiated substances (excluding squalene) showed almost no lipid peroxide level, and only non-irradiated squalene displayed a slightly raised lipid peroxide level.

Significant increases in the lipid peroxide level of squalene and oleic acid were observed after 18 h UVA-irradiation, but no significant changes in those of tetradecane, isopropyl myristate, squalane and liquid paraffin. The ratio of the lipid peroxide levels after and before UVA-irradiation was much higher for squalene than for oleic acid.

Comedo formation

Three of six non-irradiated substances (oleic acid, tetradecane and isopropyl myristate) produced very large comedones on the ventral skin of rabbit ears after 2 weeks' treatment, but squalene, squalane and liquid paraffin did not (Table 1, Figs 2 and 3).

No significant differences in the degree of the follicular hyperkeratosis or comedo formation

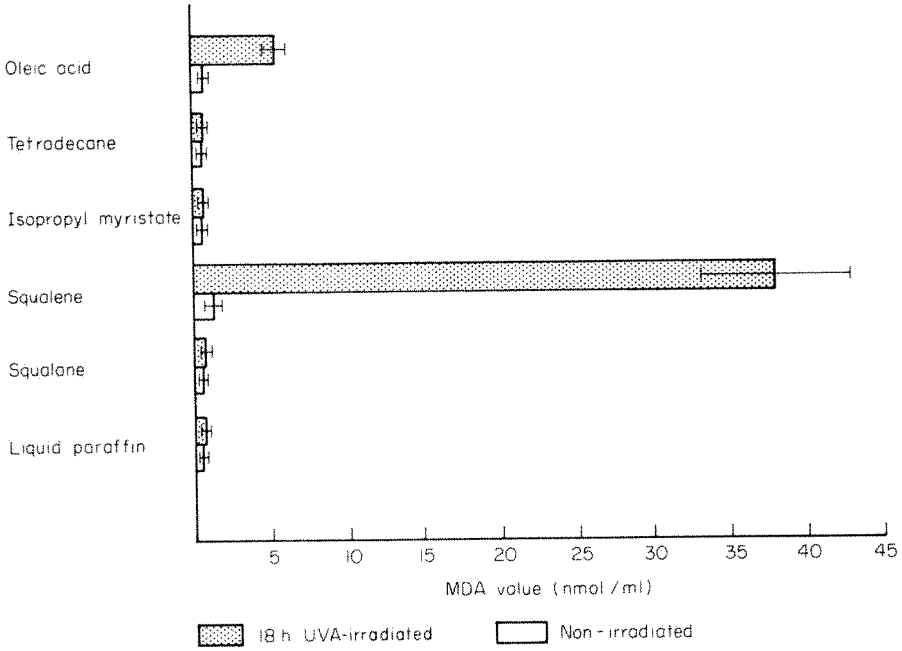

FIGURE 1. Lipid peroxide levels of six 18 h UVA-irradiated and non-irradiated substances. Each column represents a mean and a standard deviation of three determinations.

TABLE 1. Comedogenicity of UVA-irradiated and non-irradiated tetradecane, isopropyl myristate, squalane and liquid paraffin

		Average diameters (mm)*	
Substances	Days	Non-irradiated	UVA-irradiated
Tetradecane	0	0·17 ± 0·03	0·17 ± 0·03
	8	0·49 ± 0·05	0·51 ± 0·04
	14	0·78 ± 0·06	0·82 ± 0·07
Isopropyl myristate	0	0·16 ± 0·03	0·16 ± 0·03
	8	0·58 ± 0·05	0·61 ± 0·05
	14	0·68 ± 0·05	0·71 ± 0·06
Squalane	0	0·16 ± 0·02	0·16 ± 0·02
	8	0·20 ± 0·03	0·25 ± 0·05
	14	0·21 ± 0·05	0·25 ± 0·05
Liquid paraffin	0	0·16 ± 0·02	0·16 ± 0·02
	8	0·21 ± 0·04	0·24 ± 0·04
	14	0·22 ± 0·04	0·24 ± 0·05

* Figures represent a mean with standard deviation.

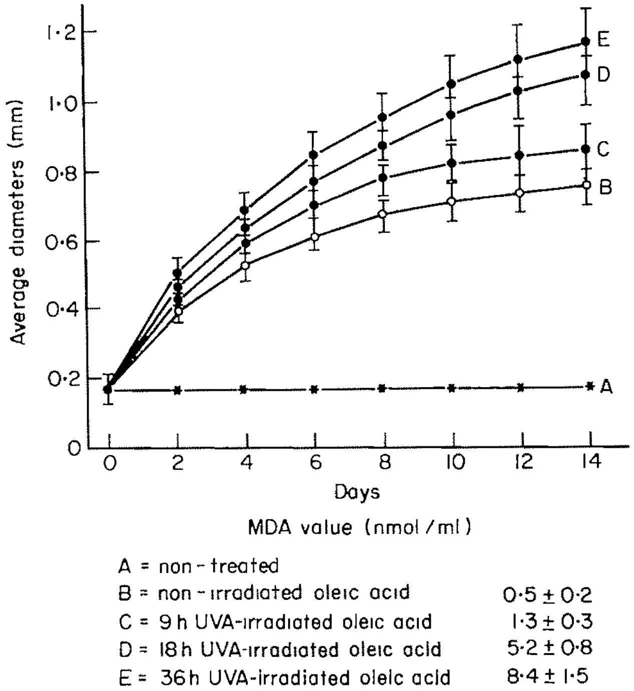

FIGURE 2. Comedogenicity of UVA-irradiated and non-irradiated oleic acid. Each point represents a mean with standard deviation MDA value represents a mean and a standard deviation of three determinations.

before and after UVA-irradiation were observed with tetradecane and isopropyl myristate (Table 1). Squalane and liquid paraffin were not comedogenic and did not become so after UVA-irradiation (Table 1). On the other hand, UVA-irradiated squalene and oleic acid showed marked comedo formation compared with the same non-irradiated substance (Figs 2 and 3).

Since the same experimental results were obtained with both conventional Japanese and New Zealand White rabbits, Table 1 and Figs 2 & 3 show only the results obtained from the latter.

Correlation between lipid peroxide level and comedo formation

A good correlation was obtained between the lipid peroxide level of squalene and oleic acid peroxides and the degree of comedo formation (Figs 2 and 3). It was noticed that as the MDA value of the peroxides was elevated, the capacity to produce comedones was increased.

Histological findings

Histological study showed marked hyperplasia and hyperkeratosis of the epithelium in the follicular infundibulum and marked proliferation of the sebaceous glands of the ear skin treated with UVA-irradiated squalene (Fig. 4). On the other hand, no remarkable histological changes except slight hyperplasia of the epidermis and slight proliferation of the sebaceous glands were observed in the ear skin treated with non-irradiated squalene (Figs 5 and 6).

The specimens from the ear skin treated with both UVA-irradiated and non-irradiated oleic acid histologically showed fairly large open comedones.

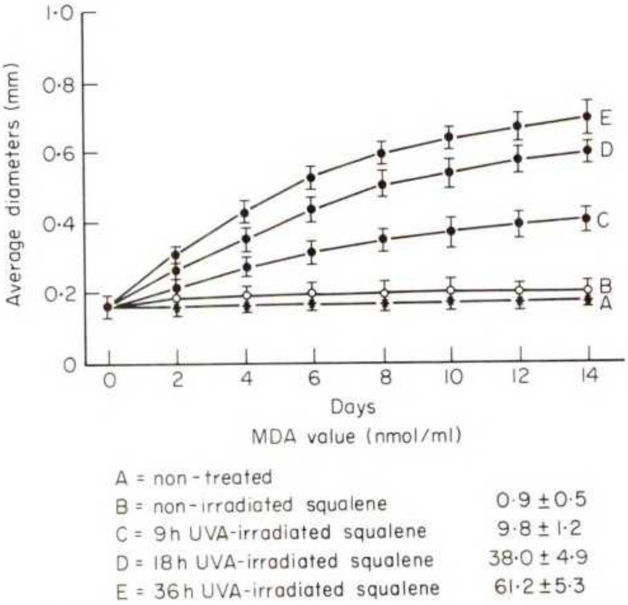

FIGURE 3. Comedogenicity of UVA-irradiated and non-irradiated squalene. Each point represents a mean with standard deviation. MDA value represents a mean and a standard deviation of three determinations.

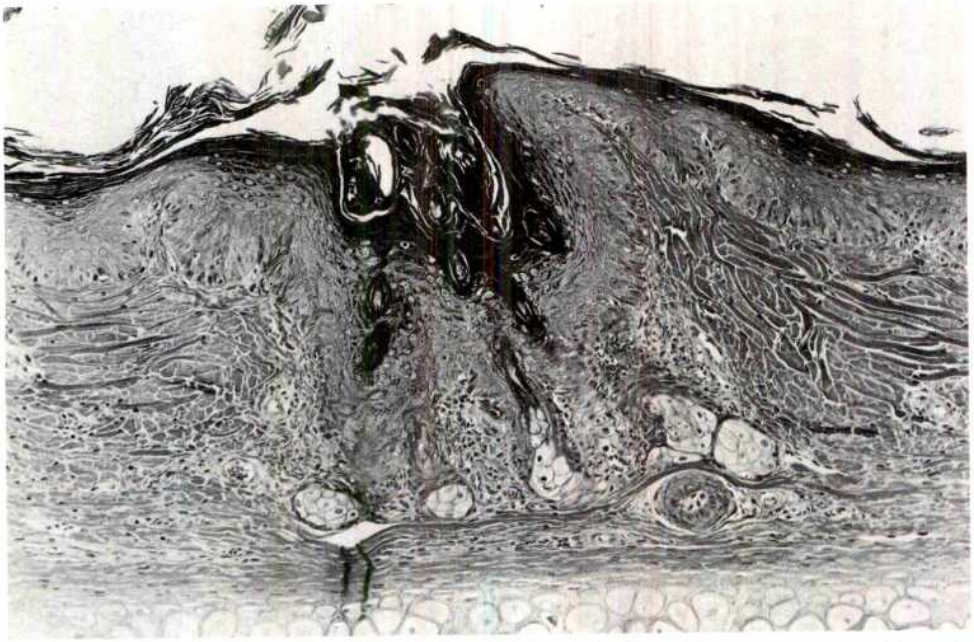

FIGURE 4. Light micrograph of the ear skin of New Zealand White rabbit treated with 18 h UVA-irradiated squalene after 2 weeks, showing marked hyperplasia and hyperkeratosis of the epithelium in the follicular infundibulum and marked proliferation of the sebaceous glands (× 124).

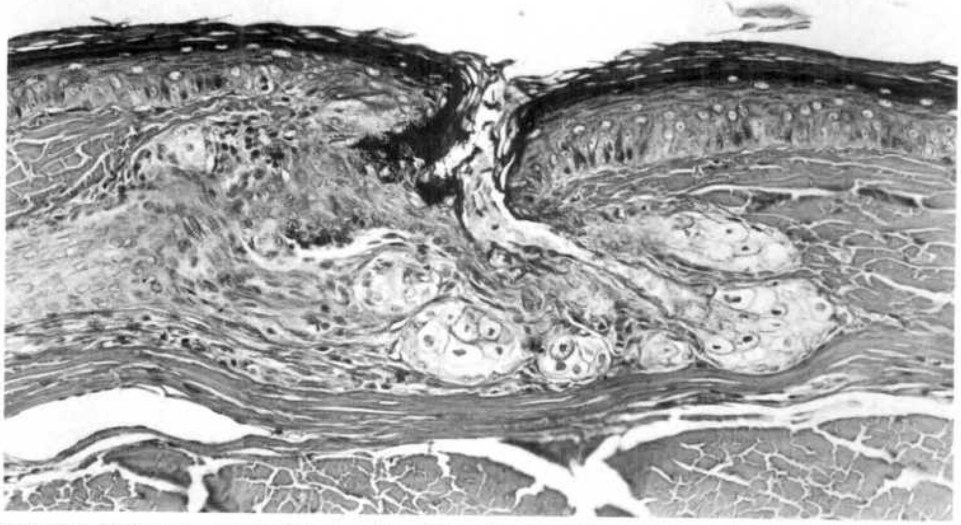

FIGURE 5. Light micrograph of the ear skin of New Zealand White rabbit treated with non-irradiated squalene after 2 weeks, showing no remarkable histological change except slight hyperplasia of the epidermis and slight proliferation of the sebaceous glands (× 196).

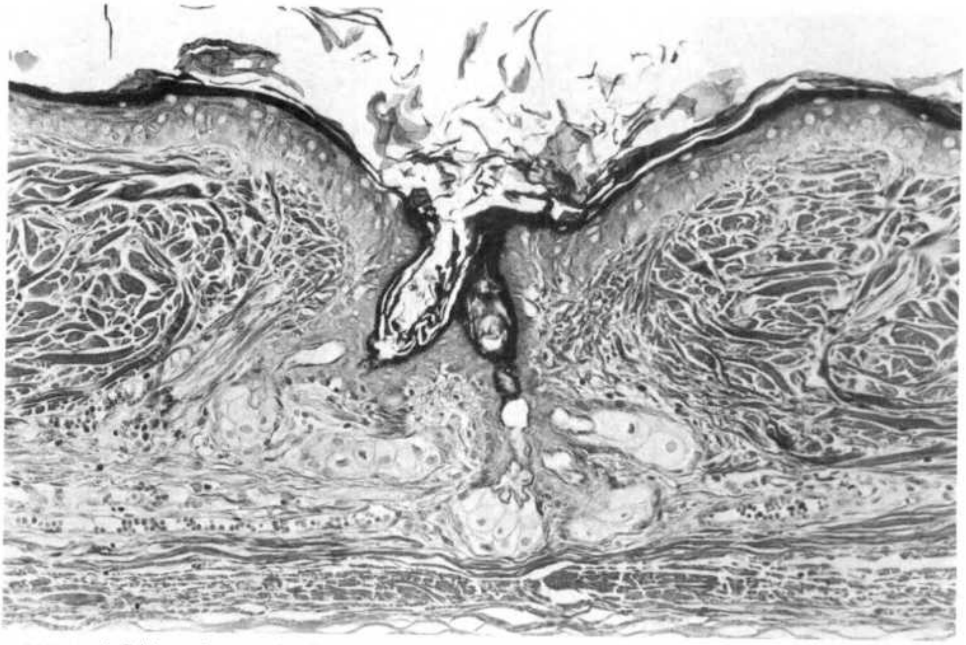

FIGURE 6. Light micrograph of non-treated control ear skin of New Zealand White rabbit (× 196).

DISCUSSION

The hypothesis that the free fatty acids might play an important role in the pathogenesis of acne (Kellum, 1968) has been generally accepted. However, Fulton, Weeks and McCarty (1975) made an extensive study of bacterial lipases and they developed drugs which act as specific inhibitors of these lipases. Such drugs, when administered to acne patients, reduced the free fatty acid level in the surface lipids, but failed to improve the clinical severity of the acne. Thus, the role of the free fatty acids in comedogenesis has remained obscure (Wheatley, 1979).

The present studies on the rabbit ear model showed that while squalene itself was scarcely comedogenic, squalene peroxides were highly comedogenic. Both oleic acid and its peroxides were able to induce fairly large comedones. A good correlation between the lipid peroxide level of squalene and oleic acid peroxides and the size of the comedones observed by a surface microscope was found. Histologically, the biopsied specimens from the ears treated with UVA-irradiated squalene showed marked hyperplasia and hyperkeratosis of the epithelium in the follicular infundibulum and marked proliferation of the sebaceous glands. However, no remarkable histological changes were seen in the ears treated with non-irradiated squalene.

These findings suggest that, although some of the free fatty acids such as oleic acid may play a role in the pathogenesis of acne, squalene in human sebum is unlikely to be a causative factor in comedogenesis, but squalene derivatives such as peroxides in the follicular infundibulum may be important in the comedo formation. Furthermore, the initiation of comedo formation might result from stimulation of the epithelium in the follicular infundibulum by these products.

Squalene peroxides can cause a strong irritation on stripped human skin and a growth inhibition in cultured human keratinocytes (Yamamoto *et al.*, 1982). Some kinds of peroxides, such as hydrogen peroxides, lauroyl peroxides and benzoyl peroxides, which are free radical-generating compounds can cause epidermal hyperplasia on the dorsal skin of Sencar mice (Klein-Szanto & Slaga, 1982). Such cytotoxic products may act as primary irritants and induce the hyperkeratosis and hyperplasia of the follicular epithelium. The thickening of the follicular epithelium may induce the obstruction of the infundibular lumina and then comedo formation.

Moreover, in this study oleic acid peroxides showed a much stronger capacity for comedo formation than oleic acid, although oleic acid in itself produced large comedones. We suggest that modification of the sebum lipids (e.g. by UVA-irradiation) may be an important factor in the pathogenesis of acne.

Mills, Porte & Kligman (1978) showed that ultraviolet irradiation enhanced the capacity of human sebum and squalene to produce comedones on the rabbit ear model. An enhancement of the comedogenicity of squalene was similarly demonstrated in man. They conjectured that in occasional patients sunbathing might aggravate acne by augmenting the comedogenicity of sebum.

On the basis of these findings and the present study, it is suggested that when human sebum and squalene are applied to both rabbits ears and the back of humans, ultraviolet irradiation might increase their lipid peroxide levels and these peroxides might irritate the epithelium in the follicular infundibulum, resulting in the augmentation in comedo formation.

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Lymphocyte subpopulations in patients with acne vulgaris

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SUMMARY

T- and B-lymphocyte subpopulations were measured in the peripheral blood of patients with varying severity of acne and in acne-free control subjects. Increased severity of acne correlated with an increased number of T lymphocytes, helper T cells and B cells, while the number of suppressor T cells remained normal and did not alter significantly. This evidence suggests that there is no lack of regulation of the immune response in acne patients.

A major factor in the inflammatory response of acne vulgaris could be the immunological reactivity of the host to antigenic stimuli, particularly the antigens of *Propionibacterium acnes*. Immunological involvement is implicit in the work of Puhvel, Hoffman & Sternberg (1966) who found elevated levels of complement-fixing antibodies in acne patients, Puhvel *et al.* (1977) who demonstrated lymphocyte transformation to *P. acnes* antigens in subjects with nodulo-cystic acne, Gowland *et al.* (1978) who showed cellular immunity (particularly in cases of severe acne) using the leukocyte migration inhibition test, and Kersey, Sussman & Dahl (1980) with their demonstration of delayed skin test reactivity. At the basis of all these immunological reactions are the subpopulations of immunologically reactive and antigen stimulus-generated lymphocytes.

It is now possible to examine lymphocytes for a range of cell surface markers especially since the advent of the OKT series of monoclonal antibodies detecting total, helper and suppressor/cytotoxic T cells (Kung *et al.*, 1979; Reinherz *et al.*, 1979a, b; Reinherz & Schlossman, 1980). Since no previous study has been reported we have carried out an investigation of T- and B-cell subpopulations in the peripheral blood of normal subjects and acne patients in an attempt to correlate changes in these populations with the severity of acne.

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TABLE 1. Normal subjects and acne patients studied

Sex	Acne grade	Number tested	Mean age ± s.e.m.	Mean acne grade ± s.e.m.
Male	Nil	15	23·46 ± 0·80	—
	Mild	11	20·30 ± 1·37	1·71 ± 0·23
	Moderate	20	20·50 ± 0·88	4·57 ± 0·18
	Severe	11	21·36 ± 1·58	7·28 ± 0·19
	Very severe	12	18·67 ± 0·91	12·56 ± 1·18
Female	Nil	14	20·50 ± 0·51	—
	Mild	24	21·13 ± 1·28	1·69 ± 0·14
	Moderate	12	22·58 ± 1·46	4·30 ± 0·25
	Severe	4	22·0 ± 4·20	7·18 ± 0·19

METHODS

Subjects

Details of the subjects studied are given in Table 1. Normal subjects were free of acne with no immediate past history of the condition. Acne patients were receiving no treatment at the time of this investigation and had received no treatment in the previous 6 weeks. The extent of the patients' acne was assessed according to the grading method of Cunliffe (1981). Patients with scores of 0–3·0 were grouped as mild, 3·0–6·0 as moderate, 6·0–8·0 as severe and grades > 8·0 as very severe acne. In our clinic it is uncommon to see females with grades > 6·0, which accounts for the low numbers of females in the severe group and the lack of females in the very severe group.

Antisera

Orthoclone monoclonal antibodies OKT3 (total T cells), OKT4 (helper or inducer T) and OKT8 (suppressor/cytotoxic T) were obtained from Ortho Pharmaceutical Corporation. Stock antibody solutions were aliquoted and stored at −20°C. Fluorescein-conjugated goat anti-mouse serum (Meloy) was diluted (1:30) in phosphate-buffered saline (PBS) pH 7·2 immediately before use.

Behring fluorescein-conjugated anti-human Ig/$F(ab)_2$ fragments and IgM/$F(ab)_2$ fragments were used to label B lymphocytes. Stock solutions (1 mg/ml) in PBS were aliquoted and stored at −20°C.

RPMI-1640 supplemented with 5% (v/v) heat-inactivated calf serum and 25 mM Hepes was stored at 4°C and used as the wash fluid throughout.

Isolation of lymphocytes

The method used was essentially that of Bøyum (1968); the final concentration of lymphocytes used was 4–7 × 10^6/ml.

Labelling of T lymphocytes

Two hundred μl of lymphocyte suspension and 5 μl of monoclonal antibody solution were incubated at 4°C for 30 min with agitation every 10 min. The cells were then washed three times in wash fluid and centrifuged at 300 × *g* for 10 min at 4°C. A final suspension of lymphocytes was made in 100 μl of wash fluid. These cells were stained by incubation with 100 μl of fluorescein-labelled goat anti-mouse serum at 4°C for 30 min. The cells were then thoroughly washed in 2 ml wash fluid three times, with centrifugation in between at 300 × ***g*** for 10 min at 4°C. The lymphocytes were finally suspended in two drops of wash fluid. Tests with each antibody were duplicated and negative controls for the goat anti-mouse serum were included.

Labelling of B lymphocytes

One hundred μl of lymphocyte suspension and 100 μl of fluorescein-conjugated Ig/F(ab)$_2$ or IgM/F(ab)$_2$ antiserum were incubated at 4°C for 30 min. The cells were then washed three times in 2 ml wash fluid with centrifugation at 300 × ***g*** for 10 min at 4°C. The final pellet was resuspended in two drops of wash fluid.

Fluorescent microscopy

A total of two hundred lymphocytes per slide were counted by direct light microscopy and the percentage showing cell membrane fluorescence were counted, using a Leitz Laborlux 12 microscope with incident UV illumination. Monocytes were excluded on morphological criteria.

Total lymphocyte counts

A Coulter counter was used for total white cell counts; total lymphocytes were calculated from the latter after a differential count.

All results were analysed using Student's t test.

TABLE 2 Lymphocyte subpopulations ($\times 10^9$/l ± s.e.m.) in controls and patients with various grades of acne vulgaris

	Acne grade				
	Nil (Controls)	Mild	Moderate	Severe	Very severe
Female					
Total T cells, OKT3$^+$	1·240 ± 0·108	1·261 ± 0·112	1·705 ± 0·097	1·443 ± 0·155	
Helper T cells, OKT4$^+$	0·807 ± 0·070	0·792 ± 0·074	1·135 ± 0·080	0·820 ± 0·096	
Suppressor T cells, OKT8$^+$	0·450 ± 0·053	0·433 ± 0·048	0·565 ± 0·049	0·529 ± 0·066	
Helper/suppressor ratio	1·879 ± 0·138	1·931 ± 0·156	2·208 ± 0·220	1·704 ± 0·173	
B cells, Ig$^+$	0·323 ± 0·026	0·333 ± 0·040	0·409 ± 0·043	0·293 ± 0·034	
B cells, IgM$^+$	0·162 ± 0·019	0·149 ± 0·024	0·164 ± 0·013	0·133 ± 0·016	
Male					
Total T cells, OKT3$^+$	1·346 ± 0·088	1·420 ± 0·113	1·466 ± 0·122	1·553 ± 0·082	1·563 ± 0·109
Helper T cells, OKT4$^+$	0·788 ± 0·054	0·892 ± 0·079	0·863 ± 0·074	0·967 ± 0·056	1·057 ± 0·101
Suppressor T cells, OKT8$^+$	0·535 ± 0·055	0·536 ± 0·058	0·601 ± 0·073	0·594 ± 0·051	0·517 ± 0·036
Helper/suppressor ratio	1·649 ± 0·150	1·833 ± 0·193	1·706 ± 0·236	1·834 ± 0·203	2·176 ± 0·267
B cells, Ig$^+$	0·320 ± 0·029	0·383 ± 0·050	0·349 ± 0·032	0·430 ± 0·044	0·423 ± 0·035
B cells, IgM$^+$	0·144 ± 0·022	0·150 ± 0·019	0·181 ± 0·034	0·218 ± 0·030	0·176 ± 0·017

RESULTS

The results are shown in Table 2.

The numbers of suppressor T cells ($OKT8^+$) in the peripheral blood of male or female patients in any acne group did not differ significantly from those in control subjects ($P > 0{\cdot}1$).

In female patients with moderate acne (grades 3·0–6·0) the mean numbers of total lymphocytes, T cells ($OKT3^+$) and helper T cells ($OKT4^+$) all increased greatly ($P < 0{\cdot}01$) but in patients with acne grades > 6·0 the mean cell numbers decreased to values very little different from those in control subjects ($P > 0{\cdot}1$) although this result may suffer from paucity of numbers.

Total T- and B-cell numbers rose steadily as the acne grades increased in male patients, but this was of no significance until the acne was of the severity of grade 6·0 or more, when numbers of total lymphocytes, helper T cells ($OKT4^+$) and B lymphocytes (cells bearing Ig surface markers) were significantly higher than cell numbers in control subjects ($P < 0{\cdot}05$).

The ratio of helper/suppressor cells ($OKT4^+/OKT8^+$) in both males and females in any acne grade was similar to the ratio for control subjects ($P > 0{\cdot}1$). In those groups where there was some increase in the ratio this was in conjunction with raised T cells ($OKT3^+$) and raised helper cells ($OKT4^+$), not with decreased suppressor cell ($OKT8^+$) levels.

DISCUSSION

This study clearly shows that acne is associated with the activation of helper T cells ($OKT4^+$), i.e. those cells intimately involved in the generation of antibody production, and that any hyper-reactivity on the part of the acne patient is not attributable to a lack of suppressor T cells ($OKT8^+$), i.e. is not associated with deficient regulation.

The more severe the condition the greater the increase in numbers of circulating $OKT4^+$ cells as compared with $OKT4^+$ numbers in normal control subjects. The correlation was shown most clearly in the male acne patients. This pattern was not followed through in the female severe acne group where there was a decrease in $OKT4^+$ cells to values found in the control and mild acne subjects. This may be due to the very low numbers in the group biasing results or it may be that females with severe acne behave differently. Obviously more females in this group must be studied. However, it is tempting to postulate that in acne vulgaris the female reacts with alacrity producing a situation where the disease cannot (in most cases) progress to absolute severity and which resolves with relative ease (hence the lack of severe acne in females in the clinic), whereas the male reacts more slowly, thus encouraging a progression towards chronicity and hence in acne towards severity.

The numbers of surface immunoglobulin (Ig)-bearing cells (total B cells) also increased significantly in the severe acne groups correlating with the increased $OKT4^+$ cell numbers. These results suggest that as the acne condition becomes more severe then IgG production keeps increasing, or alternatively as IgG production increases then the acne condition becomes worse.

However, it is clear that the increased numbers of helper cells are not due to a T-cell imbalance of the acne patient's immune system since first, suppressor T-cell numbers were not significantly lower in acne patients than in normal control subjects and second, the ratio of helper/suppressor cells was not significantly different in acne patients as a group, whatever the severity of the disease, from that in normal controls. The mean value for this ratio in normal control subjects (male and female) was $1{\cdot}76 \pm 0{\cdot}10$ and is in agreement with values given in other studies (e.g. $1{\cdot}9 \pm 0{\cdot}7$, Cosimi *et al.*, 1981).

The results of this investigation argue against the involvement of an immune disorder in the aetiology of inflammation in acne.

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Pharmacology and Treatment

Oral psoralen photochemotherapy (PUVA) of hyperkeratotic dermatitis of the palms

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SUMMARY

Oral psoralen photochemotherapy (PUVA) was found to be effective in five patients with chronic hyperkeratotic dermatitis of the palms. Relapses may occur but they respond well to retreatment.

Chronic hyperkeratotic lesions on the palms can occur alone or concomitantly with widespread dermatoses. We recently examined a subset of such patients with hyperkeratotic fissure-prone plaques proximally on the palms without evidence of psoriasis elsewhere (Hersle & Mobacken, 1982). The follow-up study supported the hypothesis that they constitute a clinical entity (hyperkeratotic dermatitis of the palms, HDP). Since standard modes of treatment are generally unsatisfactory, occupational activity may be seriously impaired.

The encouraging results of PUVA therapy in other recalcitrant palmoplantar diseases, e.g. pustulosis palmoplantaris and psoriasis (Meffert *et al.*, 1977; Menné, 1976; Morison, Parrish & Fitzpatrick, 1978; Murray, Corbett & Warin, 1980), prompted us to try it in HDP. We report here on five patients with HDP who responded well to PUVA.

METHODS

Five patients with HDP were treated. The diagnostic criteria are given elsewhere (Hersle & Mobacken, 1982). Clinical details are given in Table 1. Two patients also had a similar dermatitis on the soles of their feet. Investigations including patch testing had not disclosed any aetiological factor. All patients had been treated with common topical remedies without success.

Three times a week (Monday, Wednesday and Friday), each patient ingested 8-methoxypsoralen 0·6 mg/kg body weight (Puvamet®, Draco, Sweden) 1·5 hours before exposure to UV-A radiation. The lamp source was either PUVA 180 (Waldmann, W. Germany) or UV-A–SUN

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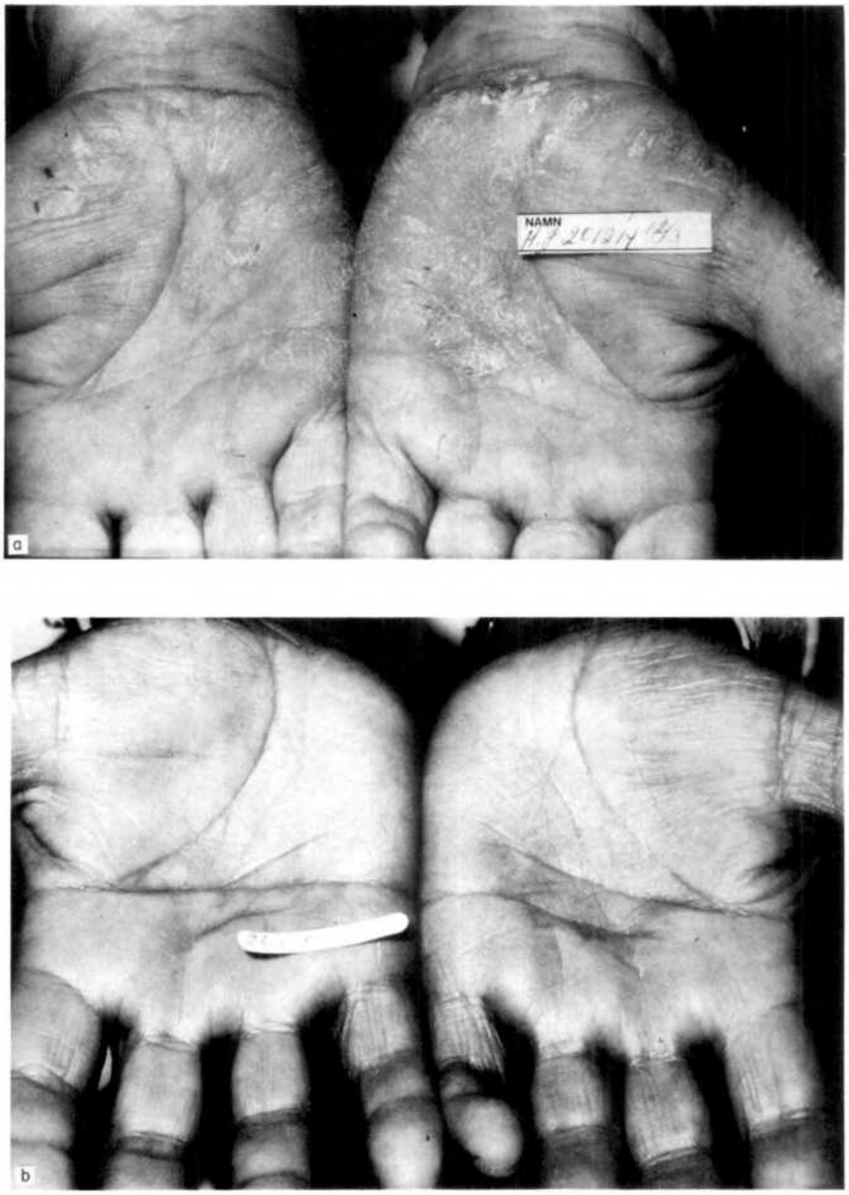

FIGURE 1. Palms of a 61-year-old woman (case 5) with hyperkeratotic dermatitis of the palms before (a) and after (b) photochemotherapy (PUVA).

2000 (Dr Mutzhas, W. Germany) (patients 1 and 2). The initial UV-A dose of 2 J/cm^2 was increased by 0·5 J/cm^2 (UV-A–SUN) to 1 J/cm^2 (PUVA 180) each treatment session up to about 10 J/cm^2. Some patients noted redness and itching on the palms, leading to temporary decrease of the UV-A dose. No maintenance treatment was given. Salicylic acid (2%) in petroleum jelly or 10% urea in an emollient base was used in the evening as a keratolytic agent. Topical corticosteroids were not used during the study.

TABLE 1. Patient response to PUVA therapy

Patient No.	Age (years)	Sex	Duration (years)	No. of treatments	J/cm^2	Results	Duration of remission (months)
1	52	F	11	19	138	Much improved	1, 5*
2	56	M	5	26	167	Cleared	3*
3	52	F	2	19	132	Much improved	1*
4	44	F	25	15	116	Cleared	6
5	61	F	11	18	85	Cleared	3

* Further treatment.

RESULTS

All patients responded to the treatment (Table 1). In three patients the lesions cleared completely for the first time in many years (Fig. 1). In two the infiltration and scaling almost disappeared and there were no more fissures. For those patients whose lesions cleared, the mean number of treatment sessions was twenty and the mean total UV-A dose 123 J/cm^2. For the improved patients the corresponding figures are nineteen treatment sessions and 135 J/cm^2. Recurrences were noted in all patients after 1 to 6 months. The lesions then gradually progressed. New treatment became necessary in three patients. The interval between treatments in these cases was 2, 8 and 9 months. The remaining two patients had only minor skin lesions after an observation period of 10 and 3 months.

The results following the second course of treatment were equally good. Two patients have since had a third course of PUVA with a similar outcome.

Three patients developed erythema of exposed skin during the first course of PUVA. A diffuse hyperpigmentation appeared on the palms and soles after the first PUVA period in one woman but had disappeared after 3 months.

DISCUSSION

This study was not controlled but the improvement of chronic lesions in all patients, irrespective of duration, clearly supports an effect of PUVA treatment. Oral administration of psoralen was preferred because of the risk of uneven penetration of topically applied photosensitizers. The fact that all patients relapsed reflects the transient nature of the responses and the inherent chronicity of the disease. The effect of repeated courses of PUVA was equally good, however.

PUVA is effective in a remarkable variety of dermatoses. Since HDP has the histopathological features of a spongiotic dermatitis (Hersle & Mobacken, 1982) it is of interest that beneficial effects have also been reported in allergic contact dermatitis and dyshidrotic eczema (Bruynzeel, Boonk & van Ketel, 1982; LeVine, Parrish & Fitzpatrick, 1981). However, the precise mechanism of action of PUVA is unknown.

Etretinate has recently been found to be very effective in HDP (Reymann, 1982) but it had to be discontinued in five out of fifteen patients because of side-effects. The present study indicates that PUVA treatment could be an effective alternative to etretinate in severe cases of HDP.

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Measurement of the response of psoriasis to short-term application of anthralin

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SUMMARY

Thirty-six patients with psoriasis were treated with short-term application of anthralin in three studies. (1) In thirteen patients 2% anthralin was applied daily to lesions on one arm for 60 min; (2) in ten patients 4% anthralin was applied daily to lesions on one-half of the body for 30 min; (3) in thirteen patients 2% anthralin was applied daily to lesions on one-half of the body for 30 min and the strength was increased to 4% and then 8% at intervals of 3 or more days, according to tolerance. Comparison was made with the response to conventional daily treatment with 0·05% to 1% anthralin. Progress was compared clinically by days to clearing, by measurements of plaque thickness with Harpenden calipers, by the frequency and severity of burning, and by patient preference. There was no significant difference in the times to clinical clearance between short-term and conventional anthralin treatments and the rate of decrease of plaque thickness was likewise similar. There was an excellent correspondence between time to reach half plaque thickness and the time for each plaque to clear clinically. There was increased burning with all the short-term treatments but the patients still preferred it. We conclude that short-term treatment with anthralin is as effective as continuous application, but that the increased burning is likely to limit its usefulness.

Recent reports suggest that psoriasis may respond to the application of anthralin (dithranol) for short periods of time (Schaefer *et al.*, 1980; Runne & Kunze, 1982). Since this would be a considerable therapeutic advance on the virtually continuous application of anthralin used in the Ingram regimen, we set out firstly to confirm the observations using objective methods (Rogers *et al.*, 1979a) as well as using clinical assessment, and secondly to optimize the effect of short-term treatment by defining the minimum time and maximum strength of anthralin which would be both effective and tolerable particularly for use by patients at home.

METHODS

Thirty-six (seventeen male, nineteen female) patients (age 14–67) were studied. Thirty had

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plaque psoriasis and six had guttate psoriasis, and in all the condition was sufficiently extensive to warrant treatment as in-patients. They were selected for the study on the basis of having lesions on the forearms suitable for the measurement of plaque thickness. The anthralin used for the short-term treatment was made up with 0·5% salicylic acid as a stabilizer (Comaish, Smith & Seville, 1971) in emulsifying ointment, this base being chosen for ease of washing-off. The ointment was applied to the lesions and removed after various intervals of time with hot water in a shower or bath. After 1–4 days tuition all the patients were able to apply the ointment themselves to most areas.

Following the short-term application and removal of the anthralin each morning, conventional treatment with concentrations of anthralin between 0·05% and 1·0% in Lassar's paste was applied to the remaining plaques of psoriasis, and left in place until the next morning (21–23 h) (as in the Ingram regimen), when it was washed off and replaced. None of the patients were treated with UV radiation or tar baths during the trial. The starting concentration of anthralin in the conventional treatment was chosen in the usual way by skilled nurses as the highest concentration the patient was thought likely to tolerate without burning. The concentration was increased stepwise at 2–7 day intervals (mean = 4·0 days ± 1·7 s.d.) according to tolerance.

Clinical response. Patients were examined each day and the response assessed by appearance and palpation. The time for clearance was recorded as the subjective assessment of the number of days taken for all (or in four cases the vast majority) of the lesions to disappear in the areas of the body treated by the different procedures.

Plaque thickness. This was measured as the difference in thickness of the psoriatic lesion and the thickness of adjacent normal skin, using suitable symmetrical lesions on each inner forearm and Harpenden calipers from which one spring had been removed to decrease compression (Rogers *et al.*, 1979a). Measurements were made on the same areas of lesional and non-lesional skin before treatment was started and then on alternate days 30–90 min after washing off the short-term treatment on one side, and before application of the conventional treatment to the other. Measurements were made until the plaque was considered to have cleared, as assessed clinically by inspection and palpation. Days to clearing (T) were recorded for each plaque, and each measurement was made without knowledge of those made previously. Using half of the pretreatment value for plaque thickness, a half clearance time ($t_{\frac{1}{2}}$) was estimated from each curve of plaque thickness against time. Rate of clearance was calculated as pretreatment plaque thickness minus final plaque thickness divided by time (T).

Burning. Patients were examined daily for burning of non-lesional skin, which was graded on a 0–3 scale of severity (1 = redness only; 2 = redness plus slight discomfort; 3 = redness plus moderate or severe pain). The number of separate sites of burning was also recorded.

Preference. At the end of treatment patient preference for either treatment was noted.

Confirmatory study: 2% anthralin applied to right arm for 60 min; conventional anthralin to rest of body

Thirteen patients were studied, eleven with chronic plaque psoriasis, and two with guttate psoriasis, all with extensive lesions on both arms. Lesions on the right arm were treated with 2% anthralin for 60 min each day and after washing off, conventional anthralin treatment was applied to lesions on the left arm and remainder of the body. Treatment was continued until all lesions on each arm had cleared clinically. Comparison of clinical response, plaque thickness and burning were made as described above for either arm.

Optimizing studies

These were done to define the shortest practicable period of application and the highest concentration for effect and tolerance.

Application time. The period taken for the application of anthralin would dictate the shortest time the material could be left on. Observation of the time taken by ten patients to apply the ointment to half the body was 3·6 minutes ± 1·1 s.d. Thus if duration of contact was critical, application time would be an unacceptably large and variable component when the time between the beginning of application and washing-off was less than 30 min. On the other hand lengthening the time would leave an increasingly unacceptable period for the patient to have to wait unclothed before washing off the ointment. We therefore decided to optimize concentrations for applications of 30 min.

Burning. Since the major limitation would be burning of clinically unaffected skin, preliminary studies were done to define the maximum tolerable concentration using 4%, 8%, 12% and 16% anthralin in emulsifying ointment applied to the uninvolved forearm skin of thirty-six patients with chronic plaque psoriasis for 30 min. Each concentration of anthralin was applied over a 2-cm diameter area and the response graded on the same 0–3 scale for burning at

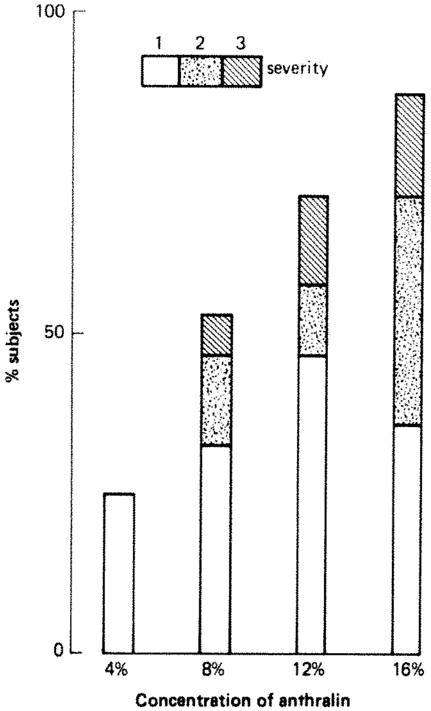

FIGURE 1. Burning response 72 h after application of anthralin (4%, 8%, 12% and 16%) for 30 min to the uninvolved forearm skin of thirty-six psoriatic subjects.

24, 48, 72 and 96 h. The maximum frequency of burning was at 72 h. At this time 25% of subjects had developed grade 1 burning with 4% anthralin whilst more than 50% had grades 1, 2 or 3 with the higher concentrations (Fig. 1). Four per cent was therefore chosen as the maximum concentration likely to be tolerated.

Anthralin (4%) applied to right half of body for 30 min: conventional anthralin to left half of the body

Ten patients were studied: nine had plaque psoriasis, one had guttate lesions. Lesions on the right half of the body were treated with 4% anthralin for 30 min, and washed off as in the first study, after which lesions on the left half were treated with conventional anthralin therapy. The treatment was repeated each day until the rash was assessed to be clinically clear as described above; lesion thickness was measured and burning assessed likewise as above.

Increasing concentrations of anthralin (2% to 4% to 8%) applied to right half of body for 30 min: conventional anthralin to left half of body

Thirteen patients were studied: three had guttate and ten had plaque psoriasis. All started treatment with 2% anthralin applied daily to the plaques on the right half of the body left on for 30 min; after 3 or more days, the concentration was increased to 4% if there was no burning, and after another minimum of 3 days, to 8%. If grade 1 burning occurred, treatment continued with the same dose and if grade 2 or 3 burning occurred, the site was left untreated for 24 or 48 h then continued using the previously tolerated concentration. Lesions on the left half of the body were treated with conventional anthralin and the lesions assessed clinically and by measurement as already described.

RESULTS

Confirmatory study: 2% anthralin applied to right arm for 60 min; conventional anthralin to rest of body

Clinical response. Eleven of the thirteen patients completed the trial and for them the mean time taken for clinical clearing of all the lesions treated with 2% anthralin for 60 min on the right arm was 11·1 days ± 3·8 s.d. compared with 11·5 days ± 3·9 s.d. for the response to the conventional therapy on the left arm ($P = < 0{\cdot}35, > 0{\cdot}30$, paired t test). One patient withdrew on day 7 because the arm treated with short-term therapy was responding less rapidly, and one patient withdrew on day 4 because of burning on short-term treatment.

Plaque thickness. Fig. 2 shows the similar decrease of plaque thickness for 60 min and conventional anthralin treatment for each patient. In all instances plaque thickness was still slightly increased when the lesions were clinically clear. A paired t test of time to clinical clearing (T), and to half clearing ($t_{\frac{1}{2}}$) of the measured forearm lesions showed no significant difference between the two treatments (Fig. 3). The overall rate of clearing of forearm lesions was likewise similar.

Burning. This was worse on the short-term treatment side throughout the trial ($P = 0{\cdot}05$ Wilcoxon rank sum test, Fig. 4a). In ten patients burning was worse on the short-term side, and in three it was worse with conventional anthralin. Most of the burns were tolerable however, only one patient having two grade 3 lesions with the short-term treatment.

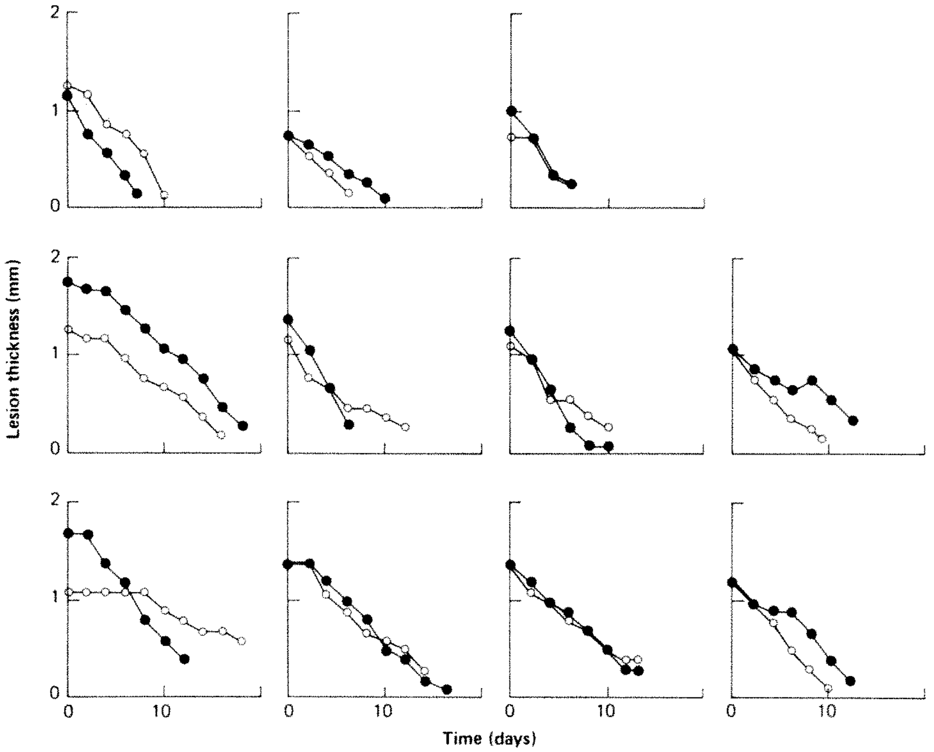

FIGURE 2. Change in forearm lesion thickness with time for 2% anthralin left on for 60 min (●) and conventional anthralin (○).

Patient preference. Eight patients preferred short-term treatment and three the conventional treatment.

Optimizing studies: 4% anthralin applied to right half of body for 30 min; conventional anthralin to left half of body

Clinical response. Eight of the ten patients completed the study. The time taken for clinical clearance of the plaques treated with short-term anthralin was 13·4 days ± 3·9 s.d. compared with 13·0 days ± 3·8 s.d. on the conventionally treated left-hand side ($P = < 0{\cdot}45, > 0{\cdot}40$; paired t test). One patient withdrew at 12 days because plaques on his abdomen and leg had failed to improve with short-term treatment whilst the lesions on the opposite side had improved with conventional therapy, and one patient withdrew at 7 days because of burning.

Plaque thickness. Decrease of plaque thickness in each individual was similar for both treatments (Fig. 5) and values for T, t½ and rate of clearing were likewise similar (paired t test, no significant difference). (Fig. 3).

Burning. As before, overall burning was worse on the short-term treated side ($P < 0{\cdot}05$ Wilcoxon rank sum, Fig. 4b). In seven patients burning was worse on the short-term treated side, equal in two and worse with the conventional treatment in one. Four patients had a total of

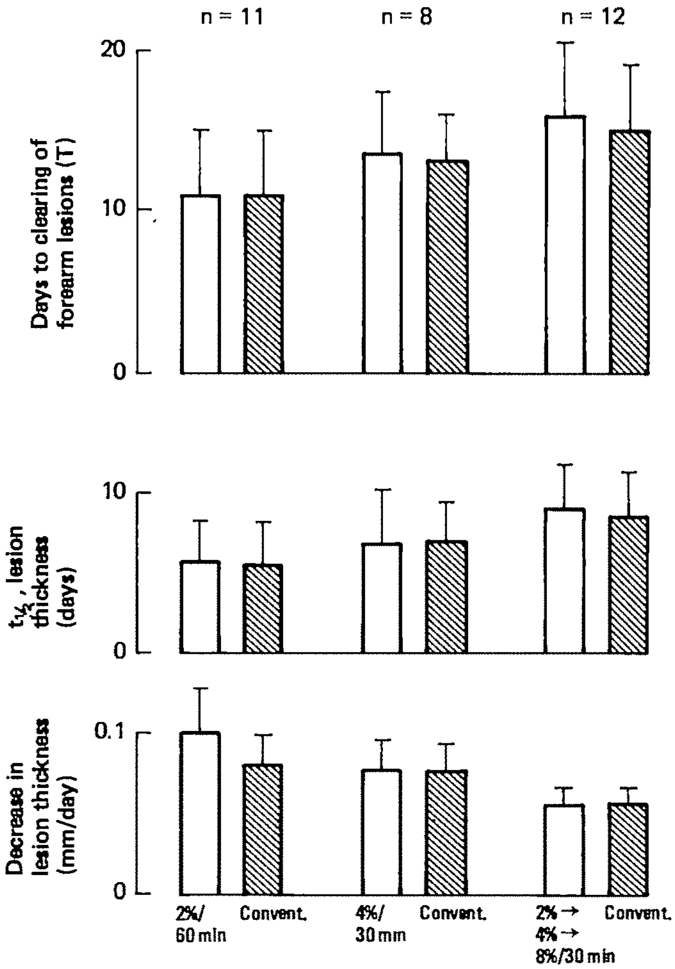

FIGURE 3. Mean ± s.d. of rate of decrease of forearm lesion thickness; of time to reach half original lesion thickness (t½); and of time to reach clinical clearing of forearm lesions (T), for each of the three short-term anthralin treatments □ and its corresponding conventional anthralin ■.

nine grade 3 lesions with the short-term treatment compared with one grade 3 lesion with conventional anthralin.

Patient preference. Five patients preferred the short term treatment, two the conventional treatment and one had no preference.

Increasing concentrations of anthralin (2% to 4% to 8%) applied to right half of body for 30 min: conventional anthralin to left half of body

Clinical response. Twelve of the thirteen patients completed the study. Only nine cleared completely on both sides with a mean time of 14·0 days ± 3·7 s.d. for the short-term treatment, 14·0 days ± 3·8 for the conventionally treated side (P = < 0·475, > 0·45; paired t test). The other

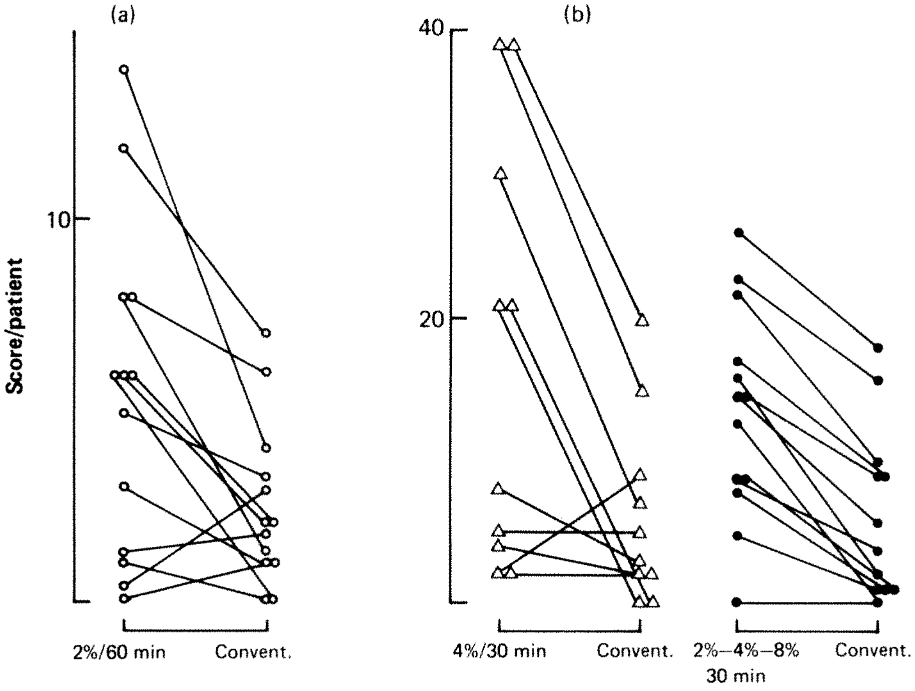

FIGURE 4. (a) Total burn scores in thirteen patients after treatment with 2% anthralin for 60 min compared with conventional anthralin $P = 0.05$ (Wilcoxon rank sum test). (b) Total burn scores in ten patients treated with 4% anthralin for 30 min compared with conventional anthralin (△), $P < 0.05$ (Wilcoxon rank sum test): total burn scores in thirteen patients treated with 2% →4% →8% anthralin for 30 min compared with conventional anthralin (●) $P < 0.01$ (Wilcoxon rank sum test).

three patients had substantial improvement with clearing at most sites except on the abdomen in two, and on the back and thigh in one. Two of these continued to use short-term treatment for 5 days after the conventionally treated side had cleared, but were still not clear by the end of this time. One patient withdrew at 13 days because large areas of plaque on his leg and abdomen had failed to improve in response to short-term treatment, although he was much better on the conventionally treated side.

Plaque thickness. Decrease in thickness of plaques was similar for either treatment (Fig. 6), with no significant difference between values for T, $t_{\frac{1}{2}}$ and rate of clearing (paired t test, Fig. 3) and they were no quicker than with the other two regimens used.

Burning. Despite the lower starting concentrations and the adjustment of higher concentrations according to apparent tolerance, burning was still worse overall with the short-term treatment ($P = < 0.01$, Wilcoxon rank sum, Fig. 4b). In twelve patients burning was worse on the short-term side and in one the scores were equal, but only two patients had grade 3 burning compared with one patient with conventional anthralin.

Patient preference. Nine preferred the short-term treatment, one conventional therapy and two had no preference.

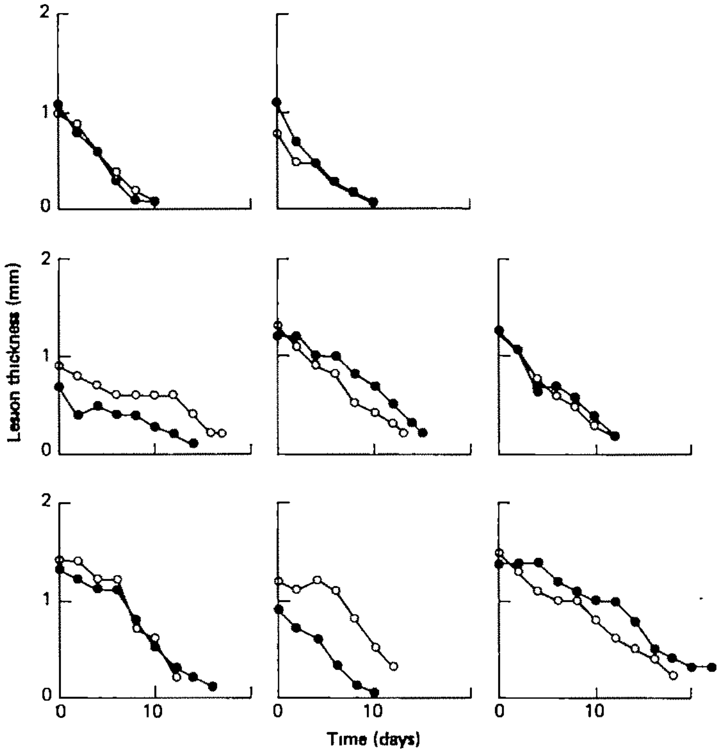

FIGURE 5. Change in forearm lesion thickness with time for 4% anthralin left on for 30 minutes (●) and conventional anthralin (○).

Relationship between clinical assessment and plaque thickness. Fig. 7 shows the excellent relationship for both short-term and conventional treatment between half clearance of forearm plaque thickness measured by calipers, and number of days to clear the forearm lesions as defined clinically ($r = 0{\cdot}86$ and $0{\cdot}84$ respectively and $P = < 0{\cdot}001$).

DISCUSSION

The present results confirm the efficacy of short-term application of anthralin, twenty-eight out of thirty-six patients clearing completely, and a further three having some improvement. Comparison with the response to conventional treatment was made clinically, and also objectively by measurement of the change in lesion thickness with Harpenden calipers. The clinical response was similar for short-term and continuous (conventional) anthralin treatment. Overall clearing times for either treatment (about 13 days) were short compared with the usually quoted figure of about 20 days for the Ingram regime for chronic plaque psoriasis (Rogers *et al.*, 1979b). This is partly explained by the fact that we included six patients with guttate psoriasis, which clears more quickly.

The objective measurements of change of plaque thickness with time likewise show little difference between the two methods of treatment, although there is variation in the shape of curves between individuals. The method for the measurement of plaque thickness has been previously validated for wealing (Cook & Shuster, 1980), eczema (Friedmann *et al.*, unpublished

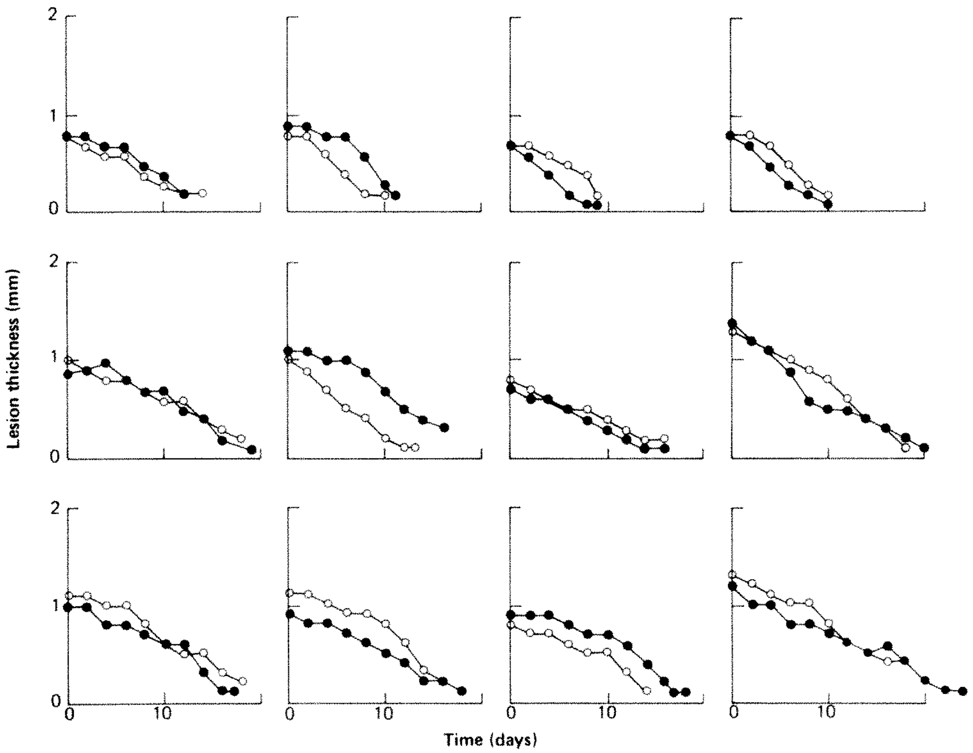

FIGURE 6. Change in forearm lesion thickness with time for 2%→4%→8% anthralin left on for 30 min (●) and conventional anthralin (○).

data) and psoriasis (Rogers *et al.*, 1979a). The problems with it are: tissue compression (small for a single reading); technique and observer error (both of which require practice); and observer bias, which can be minimized by taking each reading without knowledge of the previous one. The validity of using lesion thickness is shown by the excellent overall correlation between clinical assessment (time to clearing, T) and rate of change in plaque thickness ($t_{\frac{1}{2}}$) found with all the different treatments in all the patients studied. In addition to its objectivity, measurement of plaque thickness is more sensitive than clinical assessment and as noted previously (Rogers *et al.*, 1979a) lesions thought to be clear clinically are still measurably thickened.

Using these methods the present findings confirm the previous observations that short-term application of anthralin is effective in psoriasis and is comparable to that of conventional continuous therapy. Five of the thirty-six patients studied stopped the short-term treatment, three because of a poor response and two because of burning, but larger numbers need to be studied to permit comparison of failure rate. The main problem with the short-term treatment was burning and, not surprisingly, this was dose-related. Our regimen of gradually increasing the concentration of anthralin produced only a small decrease in burning. Although the therapeutic effect was not dependent on the concentrations we used, the burning effect was dose-dependent and hence shorter times and lower concentrations may achieve a greater therapeutic ratio. In this respect it is important to know whether the action of short-term anthralin is due to its persistence in the stratum corneum with slow absorption or to its rapid binding, e.g. to DNA.

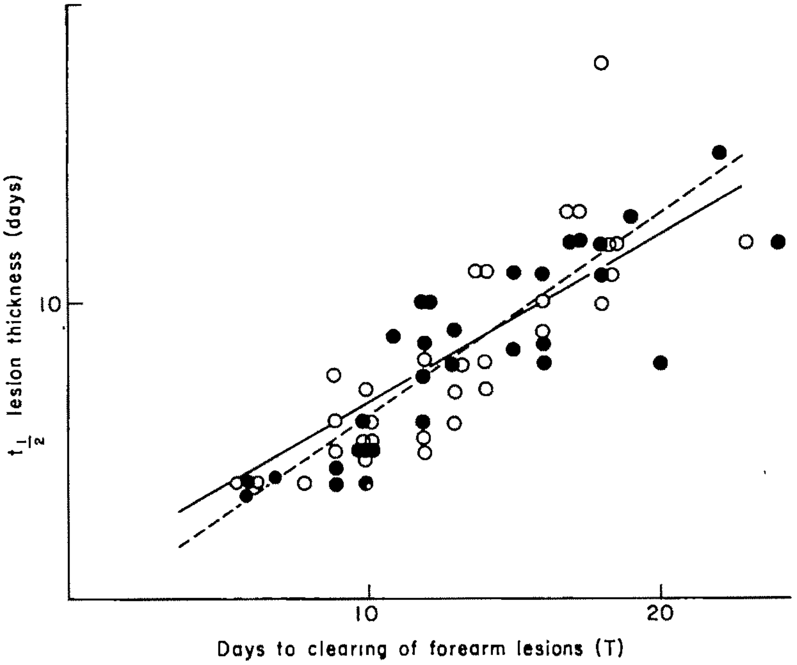

FIGURE 7. Correlation between time to reach half original lesion thickness, $t_{\frac{1}{2}}$ (objective) and time to reach clinical clearing, T, (subjective). n = 31. All short term treatments = ●, ——$r = 0·86$. All conventional treatments = ○, - - - $r = 0·84$.

It is important to be aware that systemic absorption of anthralin from the high concentrations used for short-term treatment may result in evidence of systemic toxicity, e.g. albuminuria, which does not occur with the low concentrations used conventionally. Despite this point the advantages of short-term anthralin are considerable. It is far quicker than conventional therapy and as the present study shows, can easily be carried out by the patients themselves and therefore is ideal for home treatment. It produces less staining of the skin, and because it is washed off, no staining of the clothes. Most importantly, special dressings are not required. Thus, despite the increased prevalence and severity of burning most patients preferred it to conventional therapy. For these reasons it is likely to become the treatment of choice.

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Case Reports

Hidroacanthoma simplex—a review of 15 cases

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SUMMARY

Hidroacanthoma simplex was first described in 1956, but because of its rarity there is still confusion regarding the nomenclature of this and similar entities. The histological features of fifteen cases are presented and the literature is reviewed.

Smith & Coburn (1956) introduced the term hidroacanthoma simplex to refer to superficial skin tumours originating from sweat glands which 'may be regarded as the formation of multiple sweat germs within the substance of the epidermis'. They gave the following reasons for their belief concerning the sweat apparatus origin of these lesions:

(1) The occurrence of undoubted sweat glandular differentiation in a proportion of cases.
(2) The primary origin of strictly similar neoplasia in the sweat appendages themselves.
(3) The occasional coexistence with epidermal sweat gland tumours.

They chose acanthoma as 'a general term for benign or malignant neoplasms arising mainly in the rete; their derivation from an epithelium normally composed of prickle cells', and they use simplex to refer to 'the limitation of the potential or actual differentiation to a pure sweat glandular type'.

Since 1956 there have been few references to hidroacanthoma simplex (HS) in the literature. This fact is probably due to the rarity of these lesions, as well as a confusion in terminology which may have resulted in these lesions being lumped together with similar lesions under various titles. Fifteen cases of HS are presented here and the literature is reviewed.

METHODS

Among 100,000 routine skin specimens received in our laboratory there were eleven cases of HS. In addition, four other cases were received later. Routinely prepared haematoxylin and eosin (H & E) and acid orcein-Geimsa (O & G) stained sections were available in all cases. In six of the fifteen cases additional sections were stained with periodic acid-Schiff (PAS)-Alcian Blue combination and digested PAS techniques.

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TABLE 1. Clinical data

Case number	Anatomical site	Clinical diagnosis	Sex/Age
1.	Left calf	Bowen–Basal cell epithelioma	F/65
2.	Anterior thigh	—	F/77
3.	Trunk	—	M/55
4.	Right foot	Deep mycosis	M/71
5.	—	Seborrhoeic wart	F/48
6.	Left foot instep	Irritated wart vs seborrhoeic wart vs basal cell epithelioma	F/45
7.	Right leg	—	F/66
8.	Left forearm	Bowen—Squamous cell carcinoma Superficial basal cell epithelioma	M/73
9.	Right chest	Basal cell epithelioma	M/62
10.	Forehead	Seborrhoeic wart	M/38
11.	Right lower leg	Benign keratosis	F/72
12.	Right cheek	Actinic keratosis	F/53
13.	Left chest	Seborrhoeic wart	M/66
14.	Left hip	Seborrhoeic wart	F/28
15.	Left cheek	Basal cell epithelioma— Squamous cell carcinoma	M/95

RESULTS

Clinical data of the fifteen cases of HS are summarized in Table 1. HS occurs more often on the extremities than on the trunk. The frequency of the lesions in the male and female patients is approximately equal. In the present series HS presented in the third to the tenth decades of life. There did not appear to be a characteristic clinical presentation of HS.

The most characteristic light microscopic pattern of HS is that of a rather flat acanthotic epithelial growth with nests of cuboidal to oval cells with distinct nuclei and a rim of cytoplasm (Fig. 1). The cells of this type of HS are best described as 'poroma-like'. Seven of our fifteen cases were of the poroma-like variety.

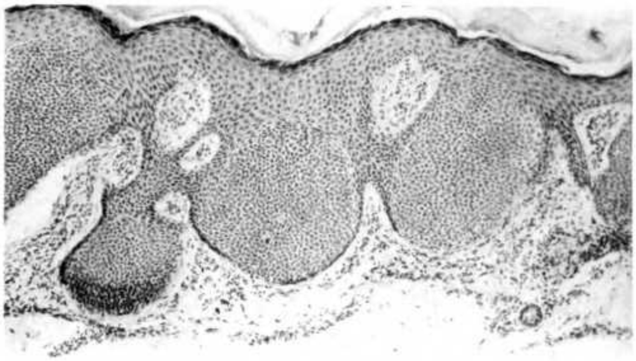

FIGURE 1. Poroma-like HS with intraepidermal nests. Note slight fibrovascular proliferation in the upper dermis (H & E, × 70).

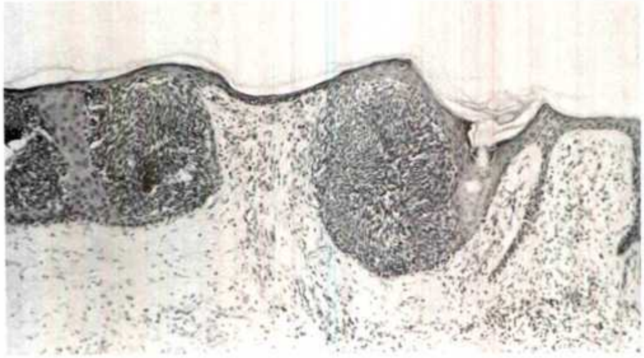

FIGURE 2. Nests of fibroblast-like appearing cells enveloped by epidermal cells (H & E, × 70).

The second histopathological variety of HS is probably the rarest, and only one of our fifteen cases would fit this type. In this variety the cells are elongated and almost fibroblast-like, forming intraepidermal nests (Fig. 2). This variety can be called the 'fibroid' type.

The third presentation has some resemblance to both the first and second varieties (Fig. 3). The HS cells forming nests in this variety are somewhat poroma-like but also show some elongation of the nuclei as one sees with fibroblasts. Two of our fifteen cases were of this 'fibroporoma-like' subtype.

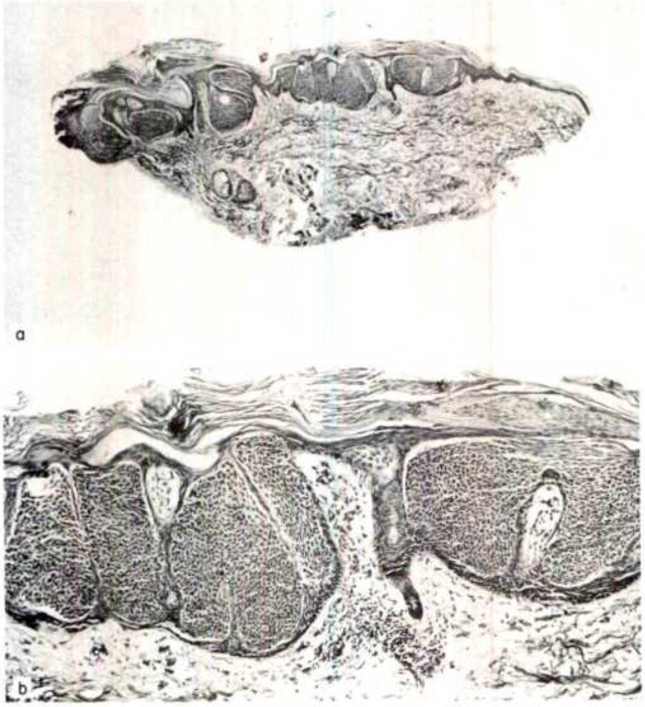

FIGURE. 3. (a) Fibroporoma-like HS (H & E, × 28). (b) HS nest showing cells with cuboidal to elongated outlines (H & E, × 70).

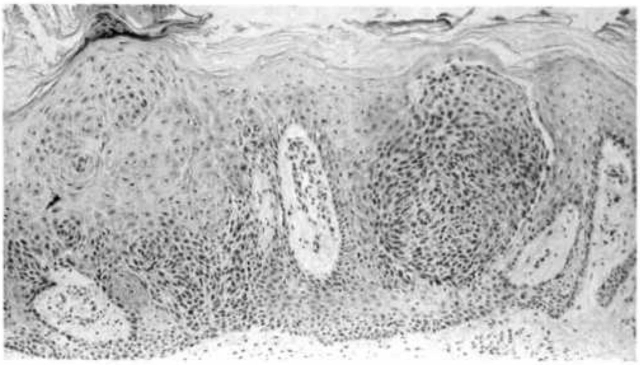

FIGURE 4. Nests of squamoid HS cells surrounded by acanthotic epidermis (H & E, × 70).

The remaining five cases showed HS cells which appeared more squamoid than basaloid (Fig. 4). This presentation can be identified as 'squamoid HS'.

In all the above varieties the HS cells are clearly different from the epidermal keratinocytes. The cells of HS may, on occasions, show eosinophilia and absence of an intact nucleus, but there is no evidence of dysplasia in benign HS. Some lesions of HS, usually of the squamoid variety, show apparently empty areas which resemble intracellular or intercellular lumina (Fig. 5).

Maturation of HS cells occurs as they reach the surface of the epidermis. Approaching the upper parts of the acanthotic growth they shrink and form parakeratotic mounds without formation of keratohyalin granules (Fig. 6). In some of the HS cells the intraepidermal nests

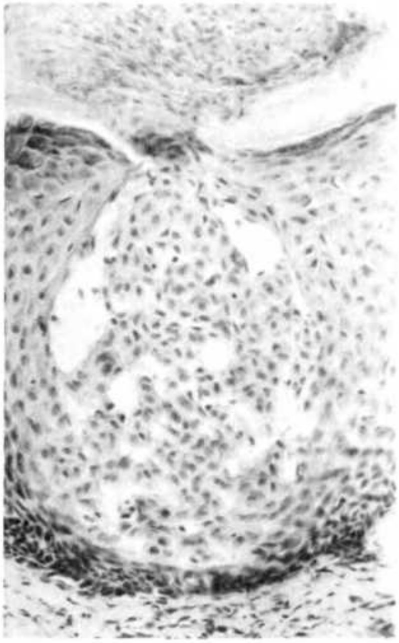

FIGURE 5. Squamoid HS showing intercellular and intracellular attempts at lumen formation (H & E, × 150).

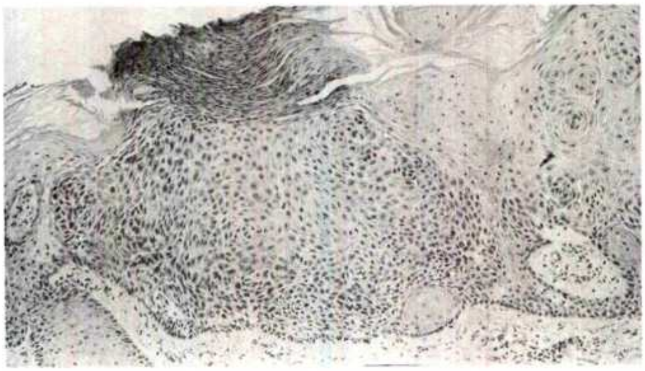

FIGURE 6. HS cells reaching the surface and shrinking to flattened parakeratotic cells with pyknotic nuclei (H & E, × 70).

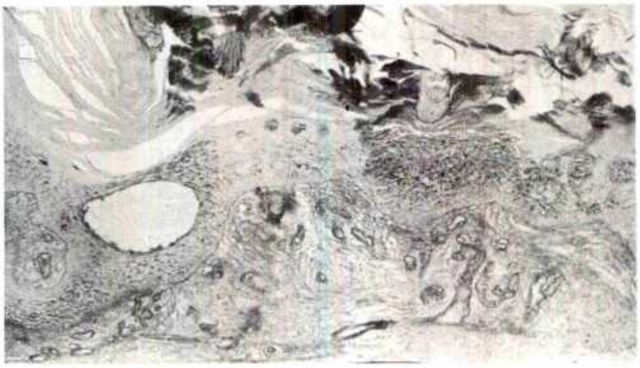

FIGURE 7. HS showing abundant glycogen. Also note the intra-nest area filled with Alcian Blue positive material. PAS-positive basement membrane is intact (AB-PAS, × 70).

show central areas of basophilic degeneration and deposition of Alcian Blue-positive material (Fig. 7).

The upper dermis beneath the tumour usually shows various degrees of fibrovascular proliferation. HS lesions are strictly intraepidermal and although there may be downward budding of epidermal rete ridges with their nests of HS cells, there is not extension of the nests into the dermis.

Traumatized lesions of HS may infrequently show cells in mitosis, but there is no cytological evidence of malignancy or invasion.

Histochemical staining of HS with Alcian blue-periodic acid Schiff and diastase-treated PAS technique demonstrates the large amount of glycogen which is usually present in HS (Fig. 7). The basement membrane below the epidermis containing HS nests is intact.

DISCUSSION

Hidroacanthoma simplex is a rare lesion and it is not well known to most dermatologists and pathologists. Pinkus, Rogin & Goldman (1956) described eccrine poroma as a tumour of the epidermal sweat duct, clearly differentiating the distal acrosyringium from the very similar surrounding epidermal keratinocytes. HS and eccrine poroma are very closely related lesions. Eccrine poroma is a tumour of the distal acrosyringium and HS comprises the proliferation of acrosyringial cells in nests throughout the involved epidermis.

It seems that there has been a reluctance to accept the existence of HS as a distinct entity. The confusion over intraepidermal epithelioma reported by Jadassohn (1926) may be responsible. Jadassohn wrote about *one* case, which probably represented a superficial basal cell epithelioma with the appearance of intrepidermal nests (Mehregan & Pinkus, 1964). This presentation is not very unusual and is familiar to many pathologists. Jadassohn (1926) also mentioned a case that Borst (1904) had reported, which was almost certainly a case of squamous cell carcinoma with dypslastic cells extending laterally and forming intraepidermal nests (Mehregan & Pinkus, 1964). The relative lack of recognition of HS as a distinct entity may also be due in part to the fact that Smith & Coburn gave their paper the subtitle 'An assessment of a selected group of intraepidermal basal cell epitheliomata'.

A list of lesions has been compiled (unpublished data) showing intraepidermal nests which I prefer to label 'Acervate Epidermal Tumours', following Cook & Ridgway (1979) who suggested acervate as the adjective emphasizing the heaped-up appearance of the intraepidermal nests. Mehregan & Pinkus (1964) have shown that many different types of lesions may manifest intraepidermal nests.

Even some authors who have accepted the sudoriferous origin of HS cells (Holubar & Wolff, 1969; Kennedy *et al.*, 1979) have preferred to avoid hidroacanthoma simplex in favour of other titles.

There have been few reports of HS since the original description by Smith & Coburn (1956). Two cases of HS were reported by Mehregan & Levson (1969). Oka, Morohashi & Nitto (1975) reported one case with light and electron microscopic findings and their case fits the squamoid HS subtype. They did not see any evidence of inter- or intra-cellular lumen formation, and on the strength of this absence, they stated that HS is related to, but not identical with, eccrine poroma. The relationship of HS to eccrine poroma is based on the proliferation of acrosyringial cells in both these lesions, but the difference between the two is the manner in which the acrosyringial cells form these growths. In HS the syringial cells proliferate over an expanse of epidermis, whereas in eccrine poroma the acrosyringial cells form an almost solid growth and replace parts of epidermis. In addition eccrine poroma has an abundant vasculofibrous stroma which is not seen in HS.

Smith & Coburn (1956) included malignant varieties of HS in their article. These malignant varieties are now better known as porocarcinoma (Mishima & Morioka, 1969), or simply as eccrine adenocarcinoma (Mehregan, Hashimoto & Rahbari, 1983), and the term hidroacanthoma simplex, without further qualification, has come to signify only the benign variety. Ishikawa (1971) reported one case of malignant HS in a Japanese woman, and Bardach (1978) also reported one case of malignant HS with malignant changes. Recently Zina, Bundino & Pippione (1982) studied a pigmented HS with areas of formation of porocarcinoma. Graham & Helwig (1972) who have written extensively about intraepidermal epithelioma, consider it a premalignant lesion related to the acrosyringial cells and sweat ductal pattern of growth.

There is general agreement that there are rather rare cutaneous lesions with intraepidermal nests, a few of which are lesions of HS. It is hoped that this paper will succeed in underlining the existence of HS as a distinct entity and emphasize the various histological forms it may assume.

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Generalized eczéma craquelé as a presenting feature of adenocarcinoma

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SUMMARY

A case of gastro-intestinal carcinoma is reported in which extensive eczéma craquelé was a presenting feature.

In 1977 Barker and Cotterill reported two cases of malignant lymphoma who presented with generalized eczéma craquelé. More recently this dermatosis has been described in association with angioimmunoblastic lymphadenopathy (van Voorst Vader *et al.*, 1979). The combination of generalized eczéma craquelé and a solid tumour has not been reported previously.

CASE REPORT

A 69-year-old man presented with a 7-week history of dysphagia, vomiting, weight loss and generalized pruritus. There was no previous history of skin disease. On examination he was clinically anaemic and had extensive erythematous eczéma craquelé involving the trunk and both legs (Figs 1 & 2). No lymphadenopathy or hepatosplenomegaly was detected.

Investigation revealed a haemoglobin of 6·9 g/l with a macrocytic film. At endoscopy he was noted to have three small gastric ulcers. Histological examination of biopsy material taken from the ulcers showed well-differentiated adenocarcinoma. Laparotomy revealed a large mass involving the small intestine which microscopically was found to consist of undifferentiated carcinoma. The pathologist (Dr G.I.Horsfield) felt these findings were consistent with a secondary deposit from an anaplastic gastric adenocarcinoma.

After laparotomy the patient's general condition deteriorated and he died 8 weeks later. During this period his eczéma craquelé was treated with topical steroid ointments with little benefit.

DISCUSSION

Eczéma craquelé is commonly seen on the shins of elderly hospital in-patients. Its appearance

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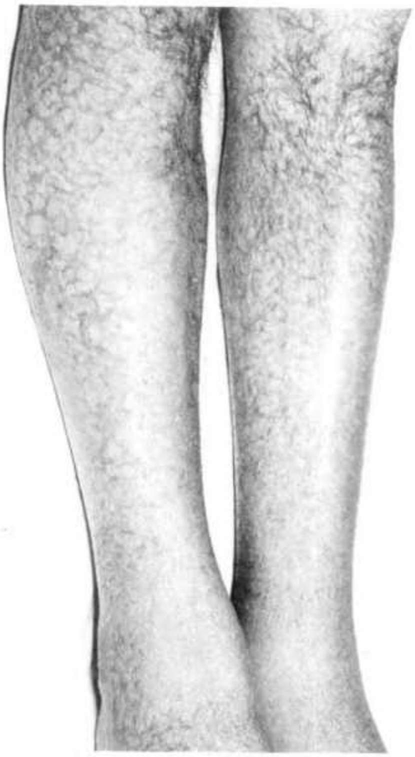

FIGURE 1. Eczéma craquelé of the legs.

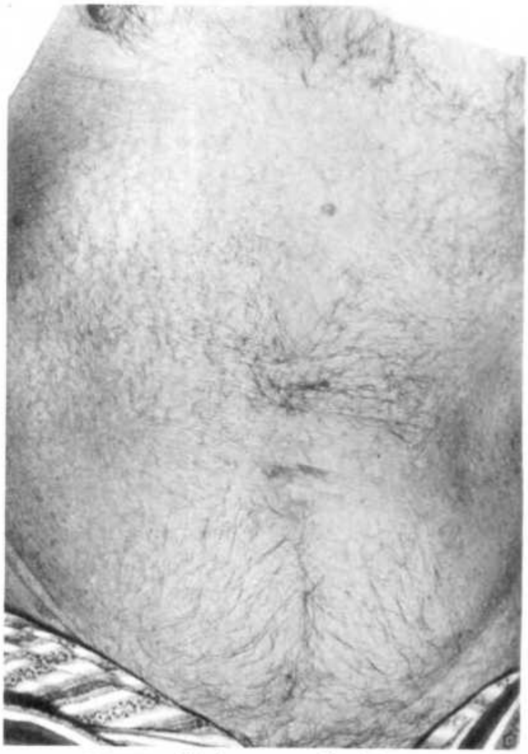

FIGURE 2. Eczéma craquelé of the trunk.

has been attributed to the degreasing effect of excessive washing. The generalized form of the disease is much rarer, develops rapidly and is resistant to treatment. It is not invariably associated with malignancy and has been linked with myxoedema (Warin 1973) and zinc deficiency (Weismann, 1979). In this case and those of Barker & Cotterill, features suggestive of malignancy were readily detected on initial examination.

I should like to re-emphasize that though rare, generalized eczéma craquelé is unmistakable and may have a sinister import. It should take its place in the increasingly long list of cutaneous signs reflecting internal malignancy.

ACKNOWLEDGMENTS

I should like to thank Dr J.Findlay and Dr D.J.Barker for permission to publish details of this case.

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British Journal of Dermatology (1983) **109**, 229–231.

Comment

VENOUS HYPERTENSION, FIBRIN AND LEG ULCERS

Dermatologists are frequently embarrassed by their inability to provide a convincing account of the pathogenesis of venous (gravitational) ulcers. For our students, we traditionally murmur the mantra 'stasis leads to anoxia and cell death', but there is little or no evidence that stasis actually occurs. It has been known for many years that the oxygen content in the femoral venous blood of a leg affected by venous hypertension is increased (Blalock, 1929; Holling, Beecher & Linton, 1938; Fontaine, 1957) and the venous blood in such limbs has a faster circulation time than normal (Piulacks & Vidal Barraquer, 1953). These observations could be explained by the development of arteriovenous shunts in the affected areas (Brewer, 1950; Guis, 1960; Haimovici, Steinman & Caplan, 1966), but the use of radioactively labelled macroaggregates or microspheres has failed to provide any evidence for such shunts (Lindemayr *et al.*, 1972; Hehne *et al.*, 1974).

An alternative explanation for these findings has recently been proposed by Browse & Burnand (1982) and this seems to be supported by a growing body of experimental data. They suggest that the high ambulatory venous pressure within the calf muscle pump is transmitted to the capillary circulation in the skin and subcutaneous tissues of the calf. This distends the local capillary bed and widens the endothelial pores, thus allowing fibrinogen molecules to escape into the interstitial fluid, where they form fibrin complexes around the capillaries. This layer of fibrin presumably forms a pericapillary barrier to the diffusion of oxygen and other nutrients which are essential for the normal vitality of the skin.

In 1956 Whimster reported that the local dermal capillary bed is enlarged in some patients with chronic venous insufficiency. This led Burnand *et al.* (1981a) to measure the size of the capillary bed and the changes in foot vein pressure during exercise in 30 normal legs and in 109 legs affected by venous hypertension. They found a good correlation between the degree of venous hypertension and the size of the capillary bed, and a causal association was confirmed experimentally in the dog hind limb (Burnand *et al.*, 1981b). The diameter of the endothelial pores increases when the intraluminal capillary pressure is raised (Shirley *et al.*, 1957; Pietra *et al.*, 1969) and in the dog, femoral vein ligation produces an increase in the fibrinogen content of the hind limb lymphatics (Leach & Browse, 1981). Burnand *et al.* (1982) have also studied patients attending a varicose vein clinic and they have shown, using phosphotungstic acid haematoxylin stains, that layers of fibrin surrounded the dermal capillaries in twenty-six legs with venous hypertension, whereas fibrin was absent in the same site in fifteen legs with clinically normal skin. They have also shown that the pericapillary fibrin deposition shows a significant correlation with dermal capillary proliferation and venous hypertension (Burnand *et al.*, 1976).

Evidence that this pericapillary fibrin probably impedes oxygen diffusion has emerged from a recent study using positron emission tomography. This technique depends on the use of the radionuclide oxygen-15 as an *in vivo* tracer. When used as $[^{15}O]_2$ it may be administered by inhalation and its tissue concentration then reflects direct oxygen utilization. It may also be given as $C[^{15}O]_2$ which provides an inert marker of tissue blood flow. The development of a system which allows the distribution of isotopes to be computed tomographically now enables regional tissue function to be studied. This technique was used by Hopkins *et al.* (1983) to study

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oxygen uptake and blood flow in the legs of sixteen patients with venous hypertension. The results showed increased blood flow and reduced fractional oxygen extraction in the diseased tissues, and this supports the hypothesis of Browse & Burnand (1982).

It seems that we must now drop the word 'stasis' from our ritual incantation for students and replace it with the phrase 'pericapillary fibrin deposition'. Yet this is not merely a question of semantics for the new concept has important implications for research and treatment. Many questions remain to be answered regarding the dynamics of the mysterious 'capillary proliferation' and fibrin deposition and the possible reversibility of these two processes. The fibrinolytic system normally breaks down fibrin to soluble degradation products, but there is some evidence that blood and tissue fibrinolytic activity are significantly depressed in patients with venous hypertension (Browse *et al.*, 1977a). It is still uncertain whether this decrease is primary, or secondary to the fibrin deposition.

Browse and his team have studied the effect of the anabolic fibrinolytic drug stanozolol (Stromba®) in lipodermatosclerosis, which is the term they apply to the painful induration of the skin and subcutaneous tissues of the lower leg secondary to venous thrombosis (also called the 'post-phlebitic leg'). In a preliminary open study they treated eight patients with stanozolol 5 mg twice daily for 3 months, and all the patients showed clinical improvement with a significant change in fibrinolytic activity towards normal levels (Jarrett *et al.*, 1976). Further experience showed that although stanozolol seemed to improve the pain and the induration in lipodermatosclerosis, it did not affect the rate of healing of the coexisting leg ulcers (Browse *et al.*, 1977b). In a double-blind crossover study of 'stanozolol plus elastic pressure bandages' versus 'placebo plus elastic pressure bandages', they compared the effects on the rate of resolution of areas of lipodermatosclerosis, and found that the addition of stanozolol for 3 months doubled the rate of resolution, and also decreased the degree of extravascular fibrin deposition in skin biopsy specimens (Burnand *et al.*, 1980). Though these results are promising, the authors advised that the drug should be used with caution. The long term safety of this drug in large numbers of patients has yet to be fully assessed. Minor abnormalities of liver function were observed in some of Burnand's patients, although these were reversible on stopping treatment. Tumours of the liver have been reported occasionally in patients subjected to prolonged treatment with anabolic steroids, although only two patients are known to have developed a liver tumour after taking stanozolol (Dr D.S. Segal, Sterling Research Laboratories). One was a child with hypoplastic anaemia, and the other was an adult with osteoporosis, and both patients had also been treated with other androgenic steroids (Henderson, Richmond & Sumerling 1973; Falk *et al.*, 1979). The use of stanozolol may also result in an alteration in the ratio of high and low density lipoproteins, but the significance of this is uncertain. Other complications include fluid retention, increased sensitivity to warfarin, and iron deficiency (Jarret, Morland & Browse, 1979; Taberner, 1983).

Although stanozolol appears to be beneficial in the management of the painful indurated post-phlebitic leg, we have as yet no direct evidence of its efficacy either in the prevention or the treatment of leg ulcers. If fibrin deposition causes tissue ulceration by blocking the diffusion of small molecules from the capillaries into the surrounding tissues, it may be possible to circumvent this blockade by supplying hyperbaric oxygen directly to the surface of the ulcerated skin. In the next Comment article, Madelene Heng (1983) reviews the possible role of hyperbaric oxygen in the promotion of healing of leg ulcers.

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Comment

LOCAL HYPERBARIC OXYGEN ADMINISTRATION FOR LEG ULCERS

The side-effects of systemic oxygen therapy which include pulmonary and central nervous system toxicity, have been studied in man (Frank & Massaro, 1979; Morgan *et al.*, 1963; Welch, Morgan and Clamann, 1963). Grand mal seizures have been reported to occur after exposure for 35–95 min at 3 atmospheres, and after 5 min at 7 atmospheres of oxygen tension (Morgan *et al.*, 1963; Welch *et al.*, 1963). Pulmonary oedema, atelectasis, consolidation, congestion, haemorrhage and fibrotic changes of the lung have been noted following systemic administration of oxygen (Frank & Massaro, 1979). Toxicity from systemic absorption of oxygen has led to consideration of local administration of oxygen for the promotion of wound healing.

It has been shown that although hyperbaric oxygen at pressures of up to 3 atmospheres, administered percutaneously, did penetrate the superficial dermis, there was no penetration of oxygen into the deep dermis (Gruber *et al.*, 1970b) with no resultant systemic toxicity (Gruber, Heitkamp & Amato, 1970a). Consequently, local percutaneous administration of hyperbaric oxygen has been successfully used by several investigators to heal ulcers, mainly those of ischaemic aetiology (Slack, Thomas & DeJode, 1966; Bass, 1971; Heng, Pilgrim & Beck, 1982) including decubitus ulcers (Gorecki, 1964; Rosenthal & Schurman, 1971; Fischer, 1970). However, there is no general agreement as to the optimum pressures of oxygen administered in the treatment of leg ulcers, and low pressures ranging from 5–15 mmHg above atmospheric pressure (Gorecki, 1964; Olejniczak, 1975) and intermediate pressures of 25–30 mmHg above atmospheric pressure (Heng, *et al.*, 1982; Fischer, 1975) have been in vogue. High pressures of 2–3 atmospheres can only be generated in modified recompression chambers (Slack *et al.*, 1966; Bass, 1971; Cooke, 1971), with attendant risks of systemic oxygen toxicity.

Treatment times have likewise varied greatly, from 15–20 min (Gorecki, 1964; Olejniczak, 1975) and 1–2 h (Slack *et al.*, 1966; Bass, 1971; Cooke, 1971) to 4–6 h (Heng, *et al.*, 1982; Fischer, 1966, 1975). It is believed that the best results might be obtained when periods of hyperbaric oxygen are interspersed with periods of tissue hypoxia when oxygen is withheld. Supporting this belief are observations, both *in vivo* and *in vitro*, that mild tissue hypoxia appears to be one of the more potent stimulators of tissue proliferation (Niinikoski, Heughan & Hunt, 1971; Goodson *et al.*, 1979; Hunt, Zelderfeldt & Dunphy, 1968). It has been demonstrated, using rabbit ear chambers, that a hypoxic tissue gradient is necessary for angiogenesis during wound healing and that when the hypoxic gradient is removed, capillary growth ceases (Knighton, Silver & Hunt, 1981). Increased angiogenetic potential has been observed in malignant tissue (Folkman, 1974) and also in the corpus luteum (Gospodarowitz & Thakral, 1977) where high oxygen gradients have been noted to be associated with increased metabolic activity and demands. In patients with intermittent claudication and in atherosclerosis increased muscle capillary diameter and density has been observed (Hammarsten *et al.*, 1980; Makitie, 1977). More clinical data is required to assess fully how these observations apply in patients with various types of leg ulcers.

We have observed (Heng *et al.*, 1982) that the ulcers treated with hyperbaric oxygen developed abundant granulation tissue which filled up the wound from the base towards the epidermal surface. It is known that in collagen synthesis, hydroxylation of lysyl and prolyl

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components is essential before glycosylated cross linkage of the collagen molecule can occur (Grant & Prockop, 1972). The reaction requires molecular oxygen as a co-factor as well as ferric ions, vitamin C and α-ketoglutaric acid. Decreased proline hydroxylation was observed in animals kept in 12% oxygen (Juva & Niinikoski, 1970) and regeneration of connective tissue collagen has been found to be increased in regions where the pO_2 was more than 15 mmHg (Gruber *et al.*, 1970a). In tissue culture, synthesis of collagen has been found to correlate with increased tension of oxygen applied to skin slices (Chvapil, Hurych & Ehrlichova, 1968). *In vitro* studies have shown that the rate of synthesis of collagen by fibroblasts was proportional to the extracellular oxygen tension, particularly in the low ranges of pO_2 reported in wounds (Niinikoski *et al.*, 1971). Likewise, the pO_2 has been shown to affect the rate of epithelial proliferation (Winter, 1970). In addition, the tensile strength of collagen in healing skin wounds and granulation tissue has been found to be increased with increased oxygen tension (Kulonen, Niinikoski & Penttinen, 1967). In bone, increased formation of callus has been found to correlate with increased tissue oxygen tension (Niinikoski, Penttinen & Kulonen, 1970). Wound tissue tonometers involving implanted silastic tubing have been useful in measuring wound oxygen tension (Niinikoski & Hunt, 1972).

The observation that a subject breathing 2–3 atmospheres of oxygen would carry about 5–6 ml of dissolved oxygen per 100 ml of blood has formed the basis for the selection of 2–3 atmospheres for the systemic administration of hyperbaric oxygen in the 1960s. However, it has been found that oxygen delivery to the healing tissue was dependent to a large extent on diffusion and not blood oxygen carrying capacity. The point should also be made that the rate of diffusion is dependent on the difference between oxygen tension in the blood supply and the tissue. Silver (1969) has reported that in some hypoxic areas, oxygen had to diffuse over distances as great as 150 microns or more to supply areas badly in need of oxygen. The presence of oedema in the tissues would obviously increase the distance separating the cells from their oxygen supply. Steep gradients of blood gases (80 mmHg for oxygen and 60 mmHg for carbon dioxide) from the capillaries to the cells have been found in wounds of resting animals breathing room air (Hunt, Niinikoski & Zelderfeldt, 1972) and injury to blood vessels and inflammation of the tissue with attendant oedema formation will, no doubt, increase the gradients still further. The delivery of oxygen directly to the proliferating epidermal and fibroblastic tissue should overcome the problem of diffusion and it may be unnecessary to administer percutaneous oxygen in the same tension as systemic oxygen to produce the same therapeutic effect in skin ulcers.

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Training in dermatology in the United Kingdom

SIR, A questionnaire concerning training in dermatology was sent in 1981 to all members of the Dowling Club resident in the United Kingdom. The membership of the Dowling Club includes not only the majority of registrars and senior registrars in training posts in dermatology, but also a large proportion of consultant dermatologists. The questionnaire stemmed from a lively discussion on training which took place during a Dowling Club 'Educational Weekend.' The prospect of a higher examination in dermatology was posed at this meeting and this evoked heated discussion from consultants and junior staff alike.

The questionnaire sought opinion on the minimum requirement for accreditation as a consultant dermatologist, the desirability and structure of a more formal training programme and the role of a higher examination in dermatology. Replies were received from 210 dermatologists. Of these 102 were consultants in N.H.S. practice, thirty-four were retired consultants, seventy-two senior registrars and registrars, one clinical assistant, one trainee general practitioner and ten respondents who did not declare their status. The opinions of those who replied to the questionnaire are as follows:

Accreditation

Ninety per cent of those who replied (irrespective of status) were in favour of maintaining as compulsory the minimum experience currently recommended by the Specialist Advisory Committee for Higher Medical Training in order to achieve accreditation as a consultant specialist in dermatology.

(a) Possession of M.R.C.P. (U.K.) before entering the speciality. (Replies stressed that possession of M.R.C.P. does not necessarily equate with clinical competence and experience in general medicine. A few suggested that M.D. or Ph.D. would make a suitable alternative.)

(b) Trainees should spend at least 2 years as a senior registrar or equivalent in a full time approved post or equivalently more in an approved part-time post. At the time of the questionnaire, only retrospective approval for training (up to a maximum of 2 years) was given to registrar posts at the discretion of the Joint Committee for Higher Medical Training (J.C.H.M.T.) Specialist Advisory Committee in dermatology (S.A.C.). Several respondents felt that the precise division between registrar and senior registrar could be more flexible so long as the total time remained the same, as occupants of such posts are almost always committed to a career in dermatology. Over 90% felt that registrar posts should be subject to approval by the S.A.C. As a result of this strong feeling, the Chairman and the Secretary of the S.A.C. approached the J.C.H.M.T. as a whole and after further negotiation and with the approval of the British Association of Dermatologists, the J.C.H.M.T. has now agreed to grant prospective approval of registrar posts in dermatology. Many felt that training should be subject to closer scrutiny by the S.A.C. (or another independent body) who should more carefully ascertain the views of juniors currently holding the posts at the time of the 5-yearly re-assessments.

Out of forty-six juniors who replied, 64% were in approved posts, 19% were in non-approved posts, and 11%, alarmingly, did not know. Only eleven out of thirty holders of approved posts knew the post became subject to re-assessment.

Training requirements

Eighty-seven per cent of consultants and 89% of registrars were in favour of a basic training, which should include a minimum of four general out-patient clinics a week for at least 4 years. The S.A.C. lays down specific training recommendations and the opinions of consultants and registrars concerning these are given in Table 1. The majority of both consultants and registrars were in favour of experience in histopathology and contact and industrial dermatitis, but several felt that attachment to a centre specialising in contact dermatitis was unnecessary. Experience in simple mycology, i.e. the preparation and

TABLE 1. Specific areas of training in dermatology laid down by the S.A.C. as (i) 'Obligatory' or (ii) 'Recommended'

	Regarded as *essential* experience by Consultants (%)	Registrars (%)
(i) *'Obligatory'*		
(a) Mycology	74	74
(b) Histopathology	90	96
(c) Patch testing and industrial dermatitis	85	96
(d) Radiotherapy	48	43
(e) Plastic surgery	55	50
(f) Venereology	53	68
(ii) *'Recommended'*		
(a) Microbiology other than mycology	53	31
(b) Genetics related to dermatology	65	38
(c) Paediatric dermatology	50	90
(d) Infectious diseases	61	60

interpretation of potassium hydroxide preparations, was regarded as essential by 74% of consultants and registrars, although a detailed knowledge of culture techniques was not necessary. A lesser proportion (up to 50%) were in favour of obligatory experience in radiotherapy, plastic surgery and venereology and the majority felt that the present recommended period of eight sessions of venereology was adequate experience. Fifty per cent of consultants and 70% of juniors felt that experience in microbiology other than mycology, genetics related to dermatology, paediatrics and infectious diseases was desirable. Over 80% of both consultants and registrars felt that a trainee should have *pratical* experience of nursing procedures (e.g. application of topical agents such as dithranol, treatment of leg ulcers, etc.)., physiotherapy procedures (such as administration of UV-B and iontophoresis) and PUVA. Only 50% of consultants and 31% of registrars regarded a period of research as relevant to the training of a dermatologist and of these the majority were in favour of only clinically-based research.

Specific questions were asked of dermatologists who had achieved consultant status within the past 5 years. Thirty-two replied, of whom twenty-seven were accredited at the time of appointment, and twenty-eight had held an approved senior registrar post. Twenty-seven considered they were adequately trained to carry out their present job, although several admitted their 'training' had been largely *ad hoc*. One consultant stressed the importance of learning managerial skills and also the approach to legal and D.H.S.S. work.

Examination

Only 16% of consultants and 8% of registrars were in favour of a higher examination in dermatology (analogous to the American 'boards') as the best way of assessing proficiency of a trainee and suitability for a consultant appointment. Only 10% of consultants and 3% of registrars thought that an examination ought to replace the accreditation system. Fears were expressed that such an examination would supplant the M.R.C.P. (U.K.) and would deny time available for research. It was suggested that such an examination, if implemented, should consist of two parts. Part 1 (to be taken after 1–2 years as a registrar) should be a theoretical examination with questions on basic sciences related to dermatology. Part 2 should be a clinical examination taken at the completion of the minimum training period. Since we analysed the results of this questionnaire the prospect of such an examination has been discussed with us by an increasing number of juniors and consultants who are in favour of a specialist examination, but there is still a strong dichotomy of opinion.

Little enthusiasm was expressed for a formal training programme akin to the residents' programme in the U.S.A., but an overwhelming majority (irrespective of status) favoured a somewhat more formal method of training with emphasis on stricter supervision of training posts by independent assessors.

It was interesting to note that there was no substantial difference of opinion between junior staff and consultants.

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Effects of short-wave ultraviolet radiation (UV-C) on delayed hypersensitivity in the guinea pig

SIR, We wish to point out that an inappropriate statistical test was used with misleading effect in the article of this title by J. Austad and Nils-Jorgen Mørk in the *British Journal of Dermatology* (1983) **108,** 63–67. The authors used the same method of analysis in their paper 'The effects of short-wave ultraviolet light (UV-B) on delayed hypersensitivity in the guinea pig' published in *Acta dermato-venereologica* (1982) **62,** 133–136) with similar but less devastating effects on the correctness of their conclusions.

Statistical analysis of the results reported in both these papers was done using Student's t-test. This test is designated for analysis of observations measured on an interval or ratio scale, while the observations reported in these papers are evidently on an ordinal scale since they are shown in terms of '+' symbols. Because of this inappropriate use of the t-test the authors are liable to claim more significant differences than actually are shown. In the second of the above two papers smaller probabilities are claimed than is justified, but the conclusions remain as stated. However in their 1983 paper, a non-parametric Kruskal-Wallis one-way analysis of variance shows that the authors are mistaken in finding that allergic responses to DNCB were still significantly reduced in one group of animals 12 days after the end of irradiation.

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Generalized pruritus as the presenting feature of bullous pemphigoid

SIR, Generalized itching in the elderly can be the result of a variety of systemic and cutaneous disorders, but many elderly patients remain undiagnosed. Severe pruritus associated with extensive blistering is a characteristic presentation of bullous pemphigoid. Recently I have followed three patients in whom generalized pruritus preceded the development of blistering by many months.

The first patient was a 69-year-old man seen in November 1979 with a 6-week history of 'terrible itching', with excoriations on the arms and legs. Examination revealed excoriated papules on the shins, forearms and shoulders but no blistering or other specific changes. A full blood count and biochemical profile were unremarkable and the pruritus improved with merely symptomatic treatment. Two months later the same patient was referred back with a 10-day history of a bullous rash on both thighs, spreading on to the trunk. Histological examination of a skin biopsy showed findings compatible with the diagnosis of bullous pemphigoid. Immunohistology of perilesional skin revealed a heavy linear deposit of C3 complement and a light deposit of IgG at the dermo-epidermal junction. The patient responded well to systemic steroid treatment and after 3 months was maintained on prednisolone 10 mg daily without any blistering.

The second case was a 77-year-old woman who presented in October 1980 with intractable generalized pruritus lasting several weeks. No blisters had ever been noted and examination revealed only excoriations and vague eczematous changes attributed to previous anti-scabies treatment. A full blood count, biochemical profile and thyroid function tests were unhelpful. In view of the experience with case 1 a biopsy of normal buttock skin was taken. Immunohistology revealed a heavy linear deposit of IgM at the dermo-epidermal junction. The patient was treated with a small dose of systemic steroids and ultimately it was found that prednisolone 7·5 mg daily controlled the pruritus. Four months later a sudden relapse occurred with extensive blistering. Immunohistology was not repeated but conventional histology revealed a subepidermal blister containing fibrin, neutrophils and plentiful eosinophils. An anti-basement membrane antibody was present in the serum at a titre of 1:100. Treatment with a higher dose of prednisolone combined with azathioprine was instituted. The blistering was rapidly controlled.

The third patient was a 65-year-old woman first seen in May 1982 with 6 weeks of generalized itching. Examination revealed multiple excoriations and vaguely lichenoid papules on the thighs and arms. A presumptive diagnosis of atypical lichen planus was made but no biopsy was performed. Topical corticosteroids appeared to control the irritation. Nearly 9 months after her original presentation the patient returned again complaining of pruritus. Groups of lichenoid papules were present on her buttocks with urticarial lesions on both thighs. Skin biopsy was performed, one of the lichenoid papules being selected. A punch biopsy of uninvolved buttock skin was also taken for immunohistology. Histological examination showed an inflammatory infiltrate in the upper dermis consisting predominantly of eosinophils: early dermo-epidermal separation was visible at the tips of two dermal papillae. Anti-basement membrane antibodies were looked for in the serum on two occasions and found in titres of 1:100 and 1:500. Immunohistology of uninvolved skin was not typical of bullous pemphigoid; granular deposits of IgM and C3 complement were present at the tips of dermal papillae.

I believe that in all these cases generalized pruritus has preceded the development of true bullous pemphigoid. The first two patients unquestionably had generalized itching months before clinical pemphigoid developed. The third patient has experienced 9 months of intractable itching but has never developed bullae. I think that in this case the histological findings and positive indirect immunofluorescence are sufficient grounds on which to base the diagnosis. The direct immunohistological findings in this case are not typical of pemphigoid and are like those described recently (Miller & Griffith, 1982) as a non-specific reaction to injury.

In their classic paper on the diagnosis and treatment of pemphigoid Sneddon & Church (1955) discuss pemphigoid prodromal eruptions. They mention an urticarial prodromal stage, which persisted for 1 to 3 weeks before blister formation, and an eczematous rash which could precede classical pemphigoid by 2 to 3 months. There may well be other 'pre-pemphigoid' eruptions. On one occasion, for example, I have seen an extensive fiery erythema precede the development of blistering by 10 days. It is possible that cases 1 and 2 were examples of eczematous prodromal eruptions although the eczematous changes in these patients were very slight and seemed more likely to be the result of over-enthusiastic anti-scabies treatment. Case 3 does not seem to fit into either category of prodromal eruptions.

It is a matter of common experience that severe irritation may precede the development of pemphigoid by a matter of days or weeks. However, the disorder does not receive regular mention in accounts of pruritus in the elderly nor is the long duration of the pre-pemphigoid pruritus appreciated.

ACKNOWLEDGMENTS

My thanks are due to Dr G.I.Horsfield, Dr B.Naylor, and Mrs Gill Alred of the Histology and Immunology Departments, Bradford Royal Infirmary.

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Bombesin—a central mediator of pruritus?

Sir, Bernstein *et al.* (1982) have proposed that endogenous opioid peptides are central mediators of the sensation of itch. We would like to point out that bombesin, another endogenous neuropeptide, may be involved in the central mediation of pruritus.

Bombesin is a tetradecapeptide which was originally isolated from frog skin (Anastasi, Erspamer & Bucci, 1971). Bombesin-like immunoreactivity has been identified in human gut (Polak *et al.*, 1976), lung (Wharton *et al.*, 1978) and cerebrospinal fluid (Yamada, Takami & Gerner, 1981). Bombesin is also produced in large amount by human cell carcinoma of the lung (Moody *et al.*, 1981). Interestingly, pruritus is often associated with lung carcinoma (Cunliffe, 1979).

We have found that intracerebroventricular or intrathecal (but not intravenous) administration of bombesin (0·001–1·0 μg) in rats elicits intense and long-lasting (45–60 min) scratching of the face, head and neck with the hindpaws. This scratching is *not* markedly affected when the rats are pretreated systemically with antihistamines (diphenhydramine, hydroxyzine, mepyramine). Morphine, naloxone, haloperidol and lidocaine, a local anaesthetic, also did not attenuate the scratching. Phenothiazines (chlorpromazine, methdilazine, trimeprazine) and analgesics of the benzomorphan class (e.g. ethylketocyclazocine, pentazocine) antagonized the scratching in a dose-related manner.

Several other peptides have been proposed as experimental pruritogens. For example, neurotensin, secretin, Substance P and vasoactive intestinal polypeptide all cause localized itch when injected intradermally in humans. These peptides, unlike bombesin, release histamine from mast cells (Fjellner & Hägermark, 1981; Sydbom, 1982).

In summary, we call attention to a possible role for bombesin in the central mediation of pruritus, especially pruritus that is resistant to antihistamines and naloxone.

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Zinc restores hair growth in lepromatous leprosy

Sir, We agree with Jablonska and Wolowa (*British Journal of Dermatology* 1981, **105**, 485) that oral zinc therapy affects hair growth only when given for a long duration, and not after a mere 3 months. Recently in the therapy of lepromatous leprosy we have tried oral zinc in addition to dapsone to enhance the suppressed cell-mediated immunity, and have compared it with patients treated with dapsone alone. Twenty-three cases of lepromatous leprosy were selected for this study. Serum zinc levels were low (60–90 μg/ml) in all these patients. They were divided into two groups. Group I (fourteen cases) received 220 mg zinc sulphate and 100 mg dapsone daily by mouth. Group II (nine patients) received only 100 mg dapsone daily. Eyebrows were absent in eight cases in Group I and four cases in Group II. Regrowth of eyebrows started in all the eight cases in Group I after about 6 months of therapy and full growth had occurred within 18 months, whereas in Group II, none showed regrowth of eyebrows. At the end of 18 months serum zinc levels had become normal in Group I but remained low in Group II. Our observations suggest that correction of zinc deficiency helps hair growth in lepromatous leprosy.

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British Journal of Dermatology (1983) **109,** 241–247.

Book Reviews

A Manual of Dermatology for Developing Countries. ORLANDO CANIZARES (1982) Oxford University Press. Pp. 355. Price £15.00. Also available in paperback, £5.00.

This is an excellent manual of some 350 pages. The review copy is clothed in a stout plastic coat—which it will need to protect it against the hard times it may have in those countries which, by common usage, we call 'developing'.

It is designed to offer medical students and doctors in those countries the basic knowledge needed for the diagnosis and treatment of the commonest skin diseases, and includes two chapters on leprosy and two on venereal disease.

'Developing countries' is a strange euphemism for those lands which have low annual per capita income. A good case could be made for describing the prosperous states which continually make more and more technological advances as 'developing', while those with bankrupt economies and failing technical installations are anything but developing, and many fall yet further behind in the race for progress. Most of the low income countries are in the tropical regions of Africa, Latin America and Asia, so this volume has a distinctly tropical leaning.

The author is at pains to point out that genetically and medically the terms 'black' and 'white' are meaningless and follows Coon's classification, which divides the world's population into five ethnic groups: Caucasoid, Congoid (Negroid), Mongoloid, Australoid and Capoid. He also finds it convenient to use the term 'tropical races' when referring to highly or moderately pigmented skin. Authors of numerous scientific papers who fall into the 'black' and 'white' trap, would do well to be as particular as he.

Treatments and diagnostic procedures are deliberately kept simple and inexpensive. A Practical Formulary appendix, containing many of the simple applications and multi-purpose bases put forward by Pettit, completes the book. There are some worrying printing errors here and surely it is wrong to include benzene, which is toxic, in the formula for tar paint. The British Pharmacopoeia formula is simply 10% coal tar in acetone.

Criticisms are few however. Following the work of Kenneth Mellanby most British dermatologists would not agree that scabies is acquired from fomites to any important extent, nor that surgery is indicated in deep lymphangioma circumscriptum. Following the report of a single case by Gange *et al.* (*British Journal of Dermatology* 1976, **95,** 445), does rifampicin really cause an eruption identical to pemphigus in sufficient numbers to merit mention of this rarity in such a text? And yet aromatic retinoids are not mentioned in the treatment of Darier's disease. Where is the evidence that patients with tuberculoid leprosy are not affected by dapsone resistance? Presumably due to a printing error, immunofluorescent studies in pemphigus erythematosus are said to show 'the intracellular antibody pattern of pemphigus'. I would not recognize a patient suffering from onchocerciasis from the textual description, but perhaps that is because my experience is African and not American. More and better photographs in colour will be badly needed by the intended readers, and a section on pathological and surgical diagnostic procedures would be helpful.

Mepacrine is not mentioned as a safe alternative to chloroquine in discoid LE, and chloroquine is advocated for malaria suppression starting 2 weeks before entry into an endemic area, which is not necessary.

The number of printing errors must annoy the author even more than the reader and does no credit to Oxford University Press. But then the book is actually printed in Hong Kong (is this a 'developing country'?) and set by Syarikat Seng Teik Sdn. Bhd., who never quite gets to grips with the 'phth' problem of words like aphthous, and it is surprising how many there are.

I wish this book very well and only hope that it actually reaches the intended readers, and that they can afford the hardback edition.

R.R.M.HARMAN

Androgens and Anti-androgen Therapy. Edited by S.L.JEFFCOATE (1982) Chichester: John Wiley. Pp. 188. Price £18.30.

This well put together book, which originated from lectures given at a symposium at the Institute of Obstetrics and Gynaecology, centres around the use of anti-androgens, in particular cyproterone acetate, for the treatment of hirsutism.

To provide the background, the reader is advised to start at chapter 2, in which the nature and source of female androgens and their metabolism are succinctly reviewed by V.H.T.James and Ann Goodall with the style and clarity which is to be expected from them. J.M.Beazley and his co-authors follow with a comprehensive review of the miscellaneous literature about the cutaneous responses to androgens and the possible mechanisms involved. According to Beazley, 'Since one or more of the blood androgens are usually raised in "idiopathic hirsutism", it remains doubtful whether a primary increased sensitivity of hair follicles needs to be invoked: minor increases in prehormones or testosterone conversion *in vitro* could be secondary to an elevation in circulating androgens.' James is more cautious : increased androgen production by the adrenals or the ovary could be responsible, but 'A differing view is that in some women the hirsutism is the result of an increased sensitivity to androgen in the peripheral tissue.'

I.D.Cooke and R.S.Sawers provide an excellent account of the clinical features of hirsutism based on their investigation of eighty-seven patients over a period of 5 years. The pharmacology of cyproterone acetate and other anti-androgens is, quite properly, introduced by Schleusener, Albring and Neumann, who pioneered the compounds, and their clinical use is discussed by Moltz, Schwarz and Hammerstein who devised the regimens, in particular 'reverse sequential therapy' involving both cyproterone acetate and ethinyloestradiol. How does the treatment work? In the studies reported by Sawers, Randall and Iqbal, measurements were made not only of the hormonal levels but of sebaceous secretion on the forehead and of hair growth on the thigh, with the object of seeking correlations. Cyproterone acetate produced small but significant reductions in total plasma androgens, and the addition of ethinyl-oestradiol a marked rise in sex hormone binding globulin. But a 40% reduction in sebum secretion was achieved during a single cycle of cyproterone acetate alone, despite the absence of any reduction in free testosterone and with only a small drop in androstenedione. They conclude that though reductions in plasma androgens and increases in SHBG may contribute to the effect, the most important action of the combined therapy is the blocking of the response of the target organ by the cyproterone acetate.

The book ends with a short discussion by M.G.Chapman of possible side-effects of anti-androgen therapy and a summary, with some thoughts about the future, by Sir John Dewhurst. The reader may now return to chapter I, entitled 'The sebaceous glands and primary cutaneous virilism' by Sam Shuster. In the expected fashion, it is a mixture of insight and idiosyncracy, unfortunately, in this instance, mainly the latter. I agree with his view that the critical factor in idiopathic hirsutism lies in the target response and that the condition would be better known as *primary cutaneous virilism*. I accept that in contrast to the prostate, 5α-reduction of testosterone may not be mandatory for the response of the sebaceous glands, but I think his statement that dihydrotestosterone plays no role whatever is unlikely to prove valid. I do not understand or accept his argument that, if an exaggerated response to testosterone is a matter of 'gene expression' rather than 'androgen modulation', therapy with anti-androgen can never be successful. I will forbear from answering the customary attack on my own experimental methods and views about the possible roles of pituitary hormones, since it is, in any case, largely irrelevant to the subject of anti-androgens.

F.J.G.EBLING

Malignant Diseases of the Vulva. STANLEY WAY (1982) Edinburgh: Churchill Livingstone. Pp. 83, B/W illustrations.

This compact and well produced book is based on 642 cases of malignant disease of the vulva that were personally recorded and followed up by the author in his long experience of gynaecology in Newcastle upon Tyne. This mass of information has been subjected to computer analysis, in which the author has been assisted by Dr. David Guthrie and Dr Peter Philips and it is therefore a very authoritative survey, not only of prognosis but of treatment and its complications. In these aspects the book is most detailed and will be an invaluable reference for gynaecologists.

In the chapter on 'The Background to Vulval Carcinoma' the author mentions the relevance of chronic granulomatous diseases in Jamaica and the importance of industrial exposure to arsenic (in crop spraying) and bitumenous oils. He also mentions the topical use of arsenic in the past as a therapy for vulval dermatoses. He particularly stresses the role of inadequate vulval hygiene, comparing the very low rate of vulval cancer in a study of Libyan women with Muslim traditions of cleanliness and loose underclothing.

However, dermatologists may not accept uncritically the authors's views on leukoplakia, based on those of Berkeley and Bonney and with only a glancing mention of lichen sclerosis et atrophicus and no mention of Hugh Wallace's classic paper. It would be of some interest if the computer could tell us more about the frequency of lichen sclerosis found in the 57% of 505 of his cases in which 'leukoplakia' was recorded.

In all of his thirteen vulval melanomas the lymph nodes were involved and the 5-year survival rate was 18·8%. At the other end of the scale he devotes a short section to 'pre-invasive carcinomas', preferring to use this title for the several conditions that dermatologists would normally separate. From a surgical aspect this is perhaps understandable, since he recognizes that these can be treated by simpler measures—although there is no mention of cryotherapy.

D.S.WILKINSON

Illustrated Encyclopaedia of Dermatology. LIONEL FRY, FENELLA T. WOJNAROWSKA AND PARVIN SHAHRAD (1981) Lancaster: M.T.P. Press. Pp. 567. Price £19.95.

The authors state that they have endeavoured to fulfill a need, that of bridging the gap between the currently available small text books of dermatology and the large reference books, by producing the *Illustrated Encyclopaedia of Dermatology*, aimed to assist trainees, general practitioners and physicians with an interest in our specialty. The emphasis is on clinical aspects of skin disease, especially differential diagnosis and guide-lines for investigation.

An alphabetical arrangement has been chosen by the authors – a little forbidding perhaps when the general practitioner reader finds that acanthosis nigricans is the first topic of fifty-eight to be discussed. The system has largely been successful, although I felt it a great pity that dermatomyositis, lupus erythematosus, mixed connective tissue disease, morphoea and scleroderma were discussed separately; and that dyskeratotic disorders, genodermatoses, Hailey-Hailey disease and ichthyosis were somewhat artificially split.

This is a highly readable book and the facts are well presented with a layout pleasing to the eye. I was repeatedly surprised at the detail and the depth of discussion that the authors had managed in their succinct comments, but in their brevity they were prone to make sweeping statements, especially on treatment aspects. On occasions the syntax strays towards lecture notes, with verbs omitted. I particularly enjoyed the sections on drug eruptions, photosensitivity, fungal infections, hyperlipidaemias, vasculitis, and the differential diagnosis aspects of the treponemal diseases.

The 'Illustrated' component of the book requires comment. For an ageing mind (such as the reviewer's) and I feel sure for the younger readers too, the histograms of age incidence and the man (person?) symbols for sex predilection gain the visual impact intended, and the distribution pattern diagrams are helpful. Unfortunately, all the clinical illustrations are of a matt finish in black and white and many are of poor quality, and are unlikely to meet the needs of the reader.

Nevertheless I enjoyed reading this book. It imparts a great deal of knowledge, presented in an attractive, easy-to-read style. It does bridge the gap as the authors intended, but for future editions serious thought will have to be given towards improving the quality of the clinical photographs.

ROY SUMMERLY

Psoriasis. Proceedings of the Third International Symposium. Edited by E.M.FARBER AND A.J.COX (1982) New York: Grune and Stratton. Pp. 530. Price £23.20.

This is not a big book physically but the range of work it encompasses is quite staggering. Research in psoriasis seems to have grown logarithmically in the last few years and few workers, even specialists in this field, can hope to keep pace with everything going on. The International Symposia inspired and directed by Eugene Farber and his team at Stanford provide a 5-yearly opportunity to catch up with, or for us at least to become aware of, the great strides being made in both basic research and clinical aspects of this exceedingly common disease. It is both common (up to 260,000 *new* cases annually in the U.S.A.) and expensive—the total annual cost to the nation being estimated at $248 million in the U.S.A.

Much of the work reported here is of high quality, often depending on high quality technology. In as wide a sweep of the subject as the editors have attempted some chaff has been gathered with the wheat and a few papers are of distinctly lower grade or even slightly 'nutty'. But with twenty-six plenary papers, fifty-six short papers, six workshops, and seventy-five poster presentations touching every aspect of the disease, a little relief is welcome at times.

Highlights for me were Stanley Cohen's fascinating paper on the molecular regulation of cellular development and division, Harvey Baker's balanced review of antimitotic therapy and Van Scott and Yu's dip into the therapeutic future with their discussion of Vitamin B3 analogues. PUVA therapy is given a fresh airing by its devotees and the new retinoid groups of drugs are fully discussed and assessed. These clearly have great potential both alone and in combination with other therapies. This book is to be dipped into for ideas, pored over for insight and education, analysed and dissected by all those seriously involved in this subject. No dermatologist or research worker can be so learned as not to learn something new and exciting from these pages.

Thank you, Gene—here's to 1986!

J.S.COMAISH

Food Allergy. Edited by J.BROSTOFF AND S.J.CHALLACOMBE (1982) London: Saunders. Pp. 260. Price £11.75.

Dr Brostoff, reader in immunology at the Middlesex Hospital, London, and Dr Challacombe, senior lecturer in immunology and microbiology at Guy's Hospital, have produced an excellent small multi-author text on food allergy which succeeds in their aim of improving understanding of the correct methods for diagnosis and management of the condition.

For the non-gastroenterologist/immunologist the introductory chapters on basic mechanisms are models of clarity and brevity. They are followed by enormous reference lists and there is a welcome absence of overlap, often a problem with multi-author books.

The second section on end-organ effects covers the gut, eczema, asthma, rhinitis, allergy to breast milk, migraine and psychological aspects and should be required reading for dermatologists. About 15% of the entire population are food allergic and yet the vast majority of dermatologists never entertain this possibility in their routine management of patients. Indeed those academic departments who might have the time for this approach are often the least prepared to try it. This may be because of the relative difficulties in the use of objective laboratory tests, a problem which is well discussed in section 3. The least satisfactory part of the book is that on clinical methods of diagnosis and treatment, where the methods suggested are more the outcome of much pragmatic experience than double-blind scientific study. However the basic concepts of food withdrawal, food challenge and masking are clearly discussed. Masking is the phenomenon whereby the presence or recent presence of the allergen (food) in the body prevents the challenge test from working.

The final section of the book is concerned with prevention of food allergy and in particular the use of breast feeding and milk avoidance to achieve this.

This is a book which should appeal to the practising dermatologist, general practitioner and medical student. Only those who have made a particular study of food allergy could find it too elementary.

P.J.AUGUST

Sarkoidose. KARL WURM (1983) Stuttgart: Theime. Pp. 242. Price DM 148.

Dr Wurm, the editor of this well-designed and well-produced volume and the author of twenty-five of the thirty chapters, and co-author of another, is a general physician at Hochenschwand in West Germany. In his foreword he estimates that in West Germany some 2000 cases of sarcoidosis are diagnosed annually, and that there are in all over 30,000 cases in the Federal Republic. It is therefore an important disease. The epidemiology of sarcoidosis is discussed at some length and the regional variations in prevalence are analysed. In West Germany the prevalence in 1967 was 21·5 per 100,000 in Lower Saxony, and 30·5 per 100,000 in Baden-Wurtemburg. In the East German Republic the prevalence rose from 2·4 per 100,000 in 1959 to 42.6 per 100,000 in 1975.

Sarcoidosis was first reported in Japan in 1921 and the present prevalence is estimated at 5·6 per 1000,000. There was an outbreak of sarcoidosis in Northern Japan in 1972–1975 in Furano-Bekken, in which the prevalence reached 140 per 100,000.

This is essentially a practical book; it presents an up-to-date account of sarcoidosis as it affects the various organ systems, with emphasis on the clinical features and differential diagnosis. The chapter on the skin has been adapted by the editor from a chapter by the late Professor Kalkoff, in Korting's *Textbook of Dermatology*. Most of the illustrations are satisfactory and some are excellent but, at least in the reviewer's copy, a disaster has overtaken two of the pages of colour plates in the chapter on the eye.

This book can be strongly recommended to those who read German and will be useful even to those who do not as a source of references to the international literature. It is to be hoped that the publishers will commission an edition in English.

A.J.ROOK

Current Dermatological Therapy. Edited by STUART MADDIN (1982) Philadelphia: W.B.Saunders & Co. Pp. 637. Price £43.

The editor has set out to distil all the recent changes in dermatological therapy into an up-to-date, convenient and practical form. This is indeed a daunting task because not only has he had to cope with a large volume of new data but also select and present clearly and concisely the most relevant subjects for practising physicians. Whilst many of these objectives have been achieved it is at a high cost to both the style of the book and selection of subject matter.

The material is presented in two parts; the first on dermatological diseases has over 200 listed contributors and has most subjects dealt with by single authors. The second is a much shorter part on medications written by the editor and his drug consultant, Dr T.H.Brown. Each section is laudably concise but unfortunately a number of subjects have been condensed to the point where they are of very limited value. For example, the diagnosis, staging and treatment of Hodgkin's disease is covered in one page. Similarly, 'Drug Eruptions' are reduced to two pages together with a large chart of the morphology of reactions. Many of the rarer conditions have no photographs and clinical descriptions that are inadequate for those who have never seen the disease. Although this is a new book on therapy the side-effects and contra-indications for treatment are often very brief and assume extensive pre-existing knowledge. For example, the section on 'Plasma Exchange' does not discuss either the method or the role of concomitant immunosuppressive therapy adequately. No mention is made of the effects on complement and immunoglobulin levels and platelets following an aggressive exchange programme. The section on medications contains some very useful data although the cytotoxic drugs in particular are dealt with very briefly. The indexing is good and includes many of the eponyms mentioned in the text.

The book will have a limited appeal in this country since it has no obvious market. I suspect there will be insufficient clinical data on common disorders and too many rarities included for the non-specialist, whilst for many specialists there will be insufficient data and too many omissions for it to act as a reference book. In addition many may be deterred by the price tag of £43. This style of concise coverage of most subjects should, however, appeal to those about to take North American examinations.

R.J.PYE

Melanoma. Histological Diagnosis and Prognosis. VINCENT J.MCGOVERN (1982) New York: Raven Press. pp. 197.

This is another admirable book from Vincent J. McGovern, Professor of Pathology at the University of Sydney. I thought that his *Malignant Melanoma: Clinical and Histological Diagnosis* (John Wiley, New York, 1976) would be hard to beat and wondered if enough new information on the tumour had been gleaned in the last 7 years to justify a further monograph on the subject. I need not have worried on both scores. This book will be even more useful than the last one especially as it contains a lot of advice stemming from recent research.

The histogenesis of melanoma is considered in detail and his criteria for assessment of atypical junctional activity (alias premalignant melanosis) are refreshingly simple and clear-cut. The author favours Clark's histogenetic classification but accepts that melanoma on glabrous skin (covered well in a chapter of its own) constitutes an entity in its own right. There is no mention of Ackerman's 'unifying concept' of the histogenesis of melanoma but I detect some tacit support for it as some of McGovern's views appear close to those of Ackerman.

Multivariate statistical analysis may be used to determine those clinical and histological features which independently influence the prognosis of patients with melanoma. It also allows complex interactions between these variables to be unravelled. The power of this tool is seen in the two chapters on the prognostic significance of clinical and histological features of melanoma. Although these contain little new information for those working in this area they give a balanced summary which should be invaluable to surgeons, dermatologists and pathologists wishing to know current views. Incidentally McGovern remains convinced that lentigo maligna melanoma (Hutchinson's melanotic freckle melanoma) behaves differently from superficial spreading melanoma and nodular melanoma and therefore deals with it separately. Comparisons in the behaviour of this tumour between low and high incidence areas (as in most parts of Australia) are overdue as there may be significant differences.

There is not only much practical help for young pathologists struggling in this often tricky field, but many useful hints for those more experienced. The value of reticulin strains and the assessment of intracellular pigment, although covered well in his last book, are again stressed. There is a new chapter on spindle cell melanotic lesions which includes good advice on their differential diagnosis.

The high quality of the abundant photomicrographs (sometimes identical to those in his last monograph), twenty colour plates and general layout makes up for the all too frequent typographical errors.

Professor McGovern's experience of melanotic lesions is second to very few. We should all be grateful that he has made fine use of this unrivalled opportunity and has allowed us to share his observations and thoughts. They make compelling reading.

J.A.A.HUNTER

Common Office Dermatology. M.V.DAHL (1983) New York: Grune & Stratton. pp. 173. Price:$39.50.

One of the commonest causes of irritation amongst dermatologists appears to be an exposure to that increasingly common environmental hazard, the small dermatological text. Their brevity encourages omission and the inclusion of adjudged irrelevant facts can tend to provide and provoke sensitive trigger mechanisms.

This text is decidedly American in content and context but is none the worse for that, in that it provides useful insight into the patterns of practice of the American physician. Particularly the so-called primary care physician at whom, along with a mysterious group called 'other health care professionals', this book is particularly directed.

I was impressed by the easy style and common sense attitudes of Dr Dahl. As a dermatologist/immunologist he has become a well-known literary figure and it is particularly praiseworthy that he has managed to combine his research interests with good orthodox clinical practice to produce chapters in which even experienced dermatologists could usefully delve.

I particularly enjoyed his chapter on poison ivy even though it is perhaps the only part of the text virtually irrelevant to British dermatologists. The unfamiliarity of the material, the botanical aspects and, not least, the folklore, are quite irresistible. Even in this chapter the stress laid on how to deal with clothing impregnated by an allergen is an important practical point not often considered by the average physician.

The points of irritation in this text were few but potent enough to warrant getting them off my chest. I did not like (but can understand) the primacy of topical corticosteroids in the management of psoriasis. I did not think a large paragraph on dialysis in the treatment of psoriasis was appropriate and I felt that the virtual invitation to use topical acyclovir on any old 'cold sore' could well be a recipe for disaster when the emergence of resistant herpes strains is already documented. Having rid myself of the irritations I must mention the soothing balm of overwhelming common sense and good clinical dermatology; particularly the opening advice on which topical steroid to use (even if unfortunately only American trade names are used in this section), the helpful approach to atopic children without over-stressing currently fashionable aspects of food allergy, and the near admission that many chronic, non-physical urticarias may even possibly be non-allergic.

All these factors made this for me an enjoyable, almost balanced, and certainly worthwhile read. At $39 it is cheap even at our present deplorably poor exchange rate and, of course, being of American origin, the quality of the production is truly first class. I certainly intend to recommend it, albeit with some reservations, to the more trustworthy of my students.

F.ADRIAN IVE

British Journal of Dermatology (1983) **109**, 248.

News and Notices

Honorary Fellowship of the Royal Society of Medicine

Dr Hugh Wallace was recently made an Honorary Fellow of the R.S.M. (London).

Fellowship of the Royal College of Physicians (London)

The following dermatologists were recently elected F.R.C.P.: B.R.Allen, D.B.Brookes, R.H.Felix, J.J.H.Gilkes, W.A.D.Griffiths, C.J.W.Guerrier, R.S-H. Tan, A.P.Warin.

Retinoids symposium, Geneva, September, 1984

An international symposium on 'Retinoids— new trends in research and therapy' will be held in Geneva, Switzerland on September 20–22, 1984. The symposium is sponsored by the European Society for Dermatological Research. For further information contact Mrs C.Wasung, Clinique Dermatologique, Hôpital Cantonal Universitaire, CH–1211 Geneva 4.

Immunobiology of the Skin and Mucous Membranes: Candida and Herpes Infections. Pavia, September 1983

An international meeting on 'Immunobiology of the Skin and Mucous Membranes: Candida and Herpes Infections' sponsored by ESDR will be held in Pavia (Italy) on 1–10 September 1983. For further information contact Prof. G.Rabbiosi, Clinica Dermatologica, Università di Pavia, Italy

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The **JOURNAL OF THE AMERICAN ACADEMY OF DERMATOLOGY** is a professional journal devoted exclusively to the clinical and continuing education needs of the entire dermatologic community, including those family practitioners, pediatricians, and internists who are concerned with clinical manifestations of skin disease in their practice.

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gram(s)	g	minute(s)	min	molar	mol/l
kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3}g)	mg	second(s)	s	gravitational acceleration	***g***
microgram(s) (10^{-6}g)	μg	cubic millimetre(s)	mm^3	micrometre(s)	μm
nanogram(s) (10^{-9}g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12}g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^{131}I
hours(s)	h	milliequivalent	mmol		

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PL 0173/0030 PA 278/7/1

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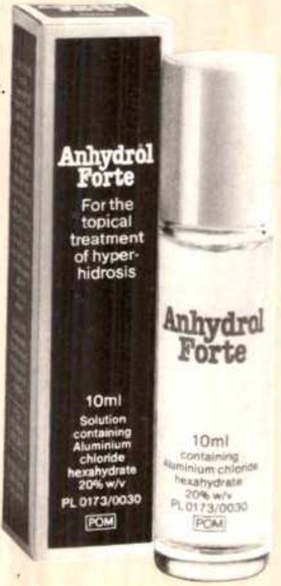
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Br J Ven Dis 1982;58:124-6

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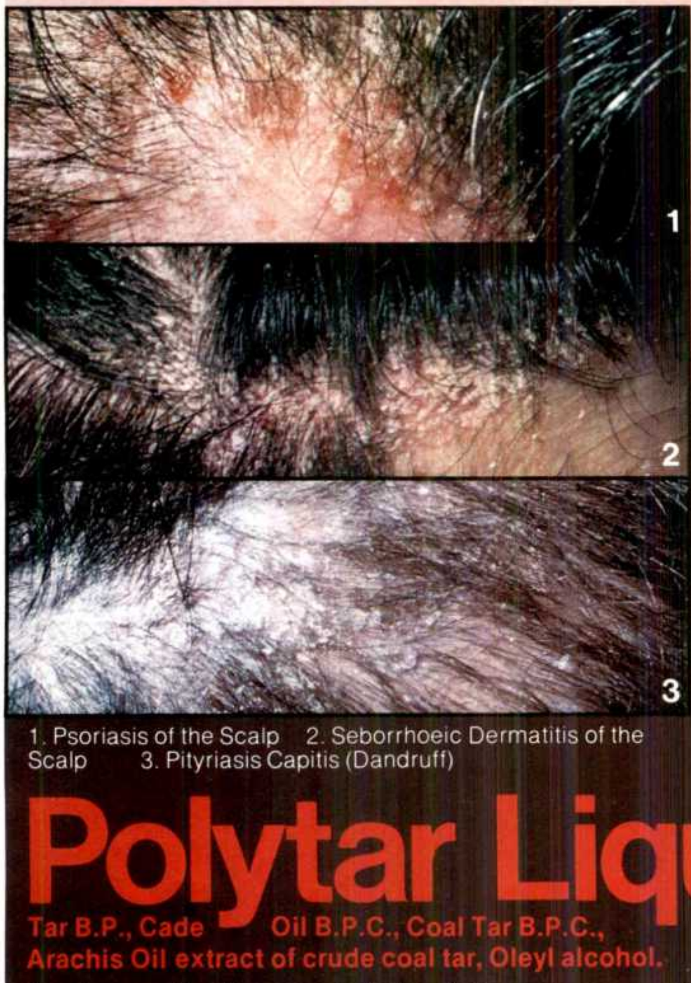
Common Disorders of the Scalp.



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Oleyl alcohol	1.00%

Polytar Plus contains 3.0% hydrolysed animal protein, in addition to the above.

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Dosage and Administration The hair should be wetted and sufficient applied to produce an abundant lather. The scalp and adjacent areas should be vigorously massaged with the fingertips. The hair should then be thoroughly rinsed and the procedure repeated.
Polytar Liquid or Polytar Plus should be used once or twice weekly.

Contra-indications, warnings, etc. Nil

Pharmaceutical Precautions Store in a cool place

Packs and Prices

	Pack	Basic NHS price
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	150ml	82p
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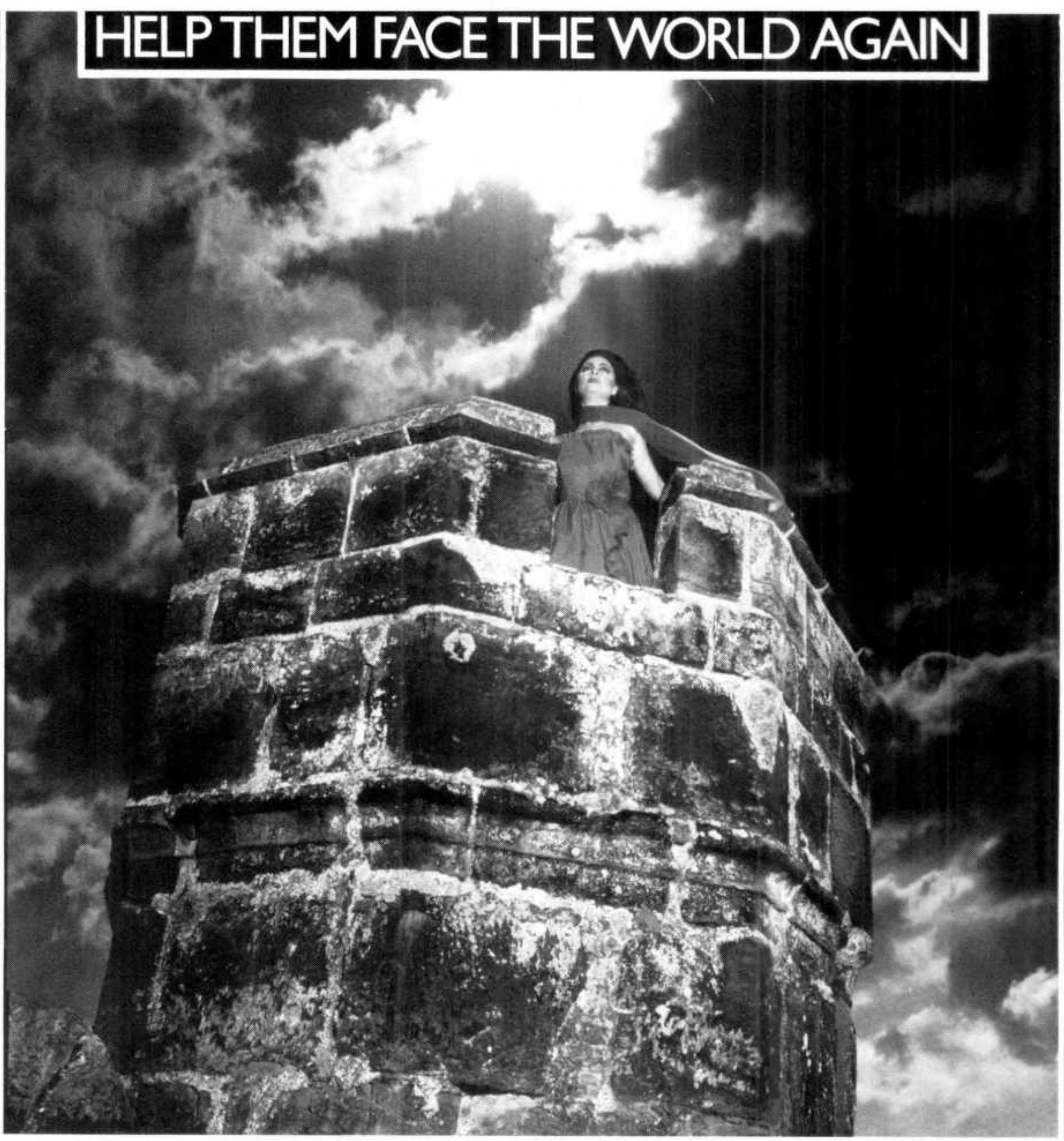

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m 75 g £1.63. Alphosyl Application PC 60 g £0.50. Alphosyl HC 30 g £1.78; 45 g £2.52. PRODUCT LICENCE NOS. 0036/5008 (Lotion). 0036/5006 (Cream). 0036/5005 (Application PC). 0036/0026 (HC).

Reference: 1. Medicine 1977; 32: 185

With acknowledgements to H.M. Bateman

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Product licence numbers

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Mims Magazine, 15th May 1982

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narked on freedom from side-effects. *Precautions:* Teratogenic studies in animals have revealed no adverse effects. However in keeping with current medical practi
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Fry L. Br Med J 1982; 284:1093—109

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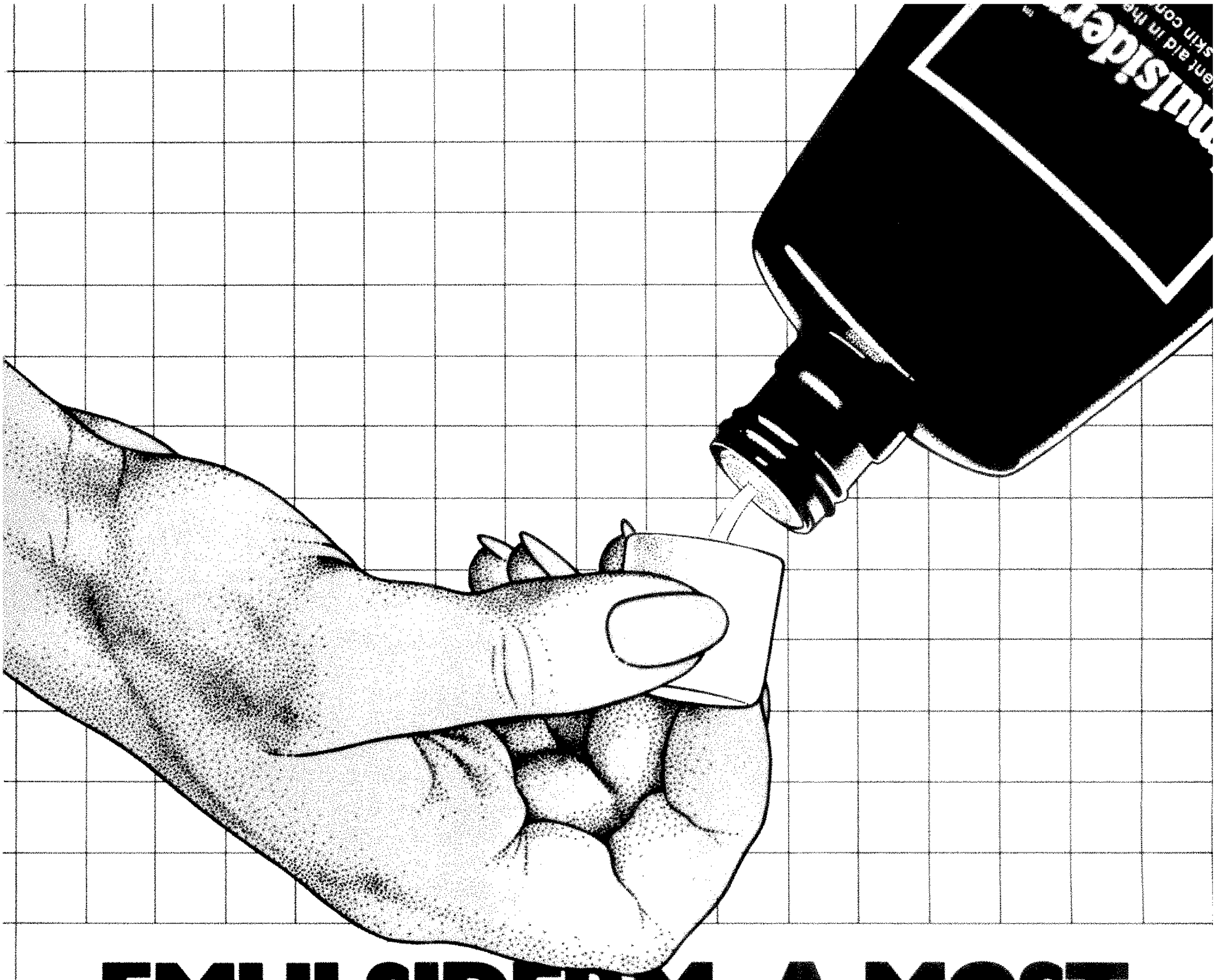

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Clinical and Laboratory Investigations

Dissociation of HLA heavy chain and light chain (β_2 microglobulin) expression on the cell surface of cutaneous malignancies

G.MAUDUIT*, M.TURBITT AND RONA M. MACKIE

Department of Dermatology, University of Glasgow

Accepted for publication 15 March 1983

SUMMARY

Biopsies from twenty-nine malignant skin tumours and four benign dermatoses and fourteen samples of normal skin were stained for cell surface membrane or cytoplasmic expression of HLA heavy chain and light chain (β_2 microglobulin) by use of the immunoperoxidase technique on frozen sections using monoclonal antibodies.

Results showed a loss of β_2 microglobulin from the surface of malignant cells in twenty-four out of twenty-nine cutaneous malignancies and the presence of HLA heavy chain in all twenty-nine primary and secondary tumours. Expression of both heavy and light chains was observed in the four benign lesions and in normal epidermis in all samples examined. This would appear to be the first report of dissociation of heavy and light HLA chain expression in various forms of cutaneous malignancy.

Beta-2-microglobulin (β_2m) is a globular protein composed of a single hundred amino-acid peptide with an intrachain disulphide bridge. It has been shown to be present on the membrane of most cells as a component of the histocompatibility antigen system (Solheim & Thorsby, 1974; Governa & Biguzzi, 1976; Forsum & Tjernlund, 1977) and is synthesized on chromosome 15.

On the cell surface β_2m is non-covalently associated with glycoproteins involved in cell–cell recognition which are coded by loci of the major histocompatibility complex (Fig. 1). In man, this complex is located in the human histocompatibility antigen (HLA) region of the 6th chromosome and includes HLA, B and C loci which code for serologically defined histocompatibility antigen associated with β_2m on the majority of cell types studied. Products of HLA-D and DR loci are not associated with β_2m.

The HLA molecule is composed of a β light chain, β_2 microglobulin (β_2m) and also a heavy chain bearing the allotypic determinants which define the HLA specificities (Grey *et al.*, 1973).

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* British Council Scholar from Hopital Edouard Herriot, Clinique Dermatologique (Pr. J. Thivolet), Lyon, France.

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A decrease or absence of β_2m portion of the HLA molecule on the surface of malignant epithelial skin tumours and a partial loss in premalignant conditions of the skin has previously been reported (Tjernlund & Forsum, 1977; Turbitt & MacKie, 1981; Korthals, Van Vinninghe & Neumann, 1981). In this study we have expanded our original work on β_2m expression on the cell surface of cutaneous malignancies to investigate the possible associated loss of HLA heavy chain expression from the surface of these cells. We have used snap-frozen tumour samples throughout the study to guard against loss of surface markers resulting from formalin fixation, and have used monoclonal antibodies raised against both β_2m and the common core of HLA antigen.

METHODS

Skin samples

Specimens comprised primary malignant melanomas (4), secondary malignant melanomas (10), basal cell carcinoma (14), squamous cell carcinoma (2), Bowen's disease (1), basal cell papilloma (3) and normal skin (14) (Table 1).

TABLE 1. Distribution of β_2 microglobulin and heavy chain HLA determinants on cell surface membranes of benign and malignant skin lesions

	β_2 microglobulin (light chain)	HLA heavy chain*
Primary melanomas (4)	2	4
Secondary melanomas (10)	2	10
Basal cell carcinomas (14)	0	14
Squamous cell carcinomas (2)	1	2
Bowen's disease (1)	1	1
Basal cell papillomas (4)	4	4
Normal skin (14)	14	14

* Cytoplasmic staining with HLA heavy chain in addition to membrane staining was present in the majority of cases.

Immunoperoxidase technique

Frozen sections were cut at 4–5 μm, collected on gelatin-coated slides and stored at −20°C until tested.

Frozen sections were washed in 0·01 M Tris-buffer pH 7·6 for 5 min, then incubated for 5 min with a 1:5 dilution of normal rabbit serum to reduce non-specific staining. This was poured off and the sections were then incubated for 60 min at 20°C with monoclonal antibodies diluted at 1:50.

(1) mouse IgG 2a antibody against human β_2m (MAS 018, Sera-Lab)

(2) mouse IgG 2a antibody against HLA A, B, C (shared determinant) (MAS 032, Sera-Lab)

After washing, sections were incubated for 45 min at 20°C with rabbit anti-mouse IgG/horseradish peroxidase conjugate 1:20 (Dakopatts, A/S Mercia Diagnostics) with 50% human serum. All antisera were diluted in 0·01 M Tris-buffer pH 7·6 with washes in Tris-saline (9 parts 0·85% NaCl, 1 part 0·01 mol/Tris/HCl pH 7·6).

The presence of the bound peroxidase enzyme was revealed by reaction with 0·03% DAB (30 mg 3 3′-diaminobenzidine tetrahydrochloride in 100 ml 0·01 M Tris-buffer pH 7·6 with 0·03% H_2O_2) for 5 min at 20°C, or 0·02% AEC (20 mg 3-amino 9-ethyl carbazole dissolved in 5 ml dimethylformamide, then 100 ml acetate buffer 0·05 M pH 4·9 with 200 μl H_2O_2 30%) for 5 min at 20°C. Sections were then briefly counterstained with haematoxylin and mounted with piccolyte resin for DAB stained sections or water mountant for AEC stained sections.

In seven cases the three stage avidin–biotin immunoperoxidase technique was used (Guedson, Termynck & Avrameas, 1979). After sectioning the slides were air-dried for 20 min, then fixed in acetone for 10 min, washed in PBS with thiomersal and immersed in 7·5% H_2O_2 PBS for 10 min to remove endogenous peroxidase staining. The sections were then incubated sequentially with monoclonal antibodies (β_2m or HLA) anti-mouse immunoglobulins/biotin conjugate 1:40 for 15 min and avidin/horseradish peroxidase conjugate 1:100 for 15 min.

All antisera were diluted in PBS with thiomersal $4{\cdot}9 \times 10^{-5}$M. Bound peroxidase was revealed as previously indicated.

Controls consisted of:

omission of primary antibody;
use of ‘irrelevant’ monoclonal antibody (e.g. OKT3, Ortho);
omission of rabbit anti-mouse immunoglobulin;
application of DAB or AEC alone.

RESULTS

A characteristic pattern of positive brown (with DAB) or red (with AEC) intraepithelial staining for both β_2m and HLA heavy chain was seen in the normal epidermis adjacent to the lesional skin of all cases with the exception of five secondary melanomas in which no epidermis was present. In fourteen additional sections of normal skin used as controls the same pattern was observed (Figs 1 and 2). Even at low dilutions (1:100 or less) there was an impression in some areas of light cytoplasmic staining with the HLA antibody in addition to the membrane staining.

In the basal cell papillomas regular uniform staining of surface membrane on all cells was observed with both β_2m and HLA heavy chain. The pattern was identical to that seen in the normal epidermis adjacent to the cases of cutaneous malignancy. Of the cutaneous malignancies all twenty-nine showed membrane staining of tumour cells with HLA heavy chain. This, however, was patchy within individual tumours and in addition to membrane staining, some cytoplasmic staining was observed in the majority of tumour cells (Figs 3–7). Only five out of twenty-nine tumour samples showed any regular membrane staining with β_2m (four melanomas and one squamous carcinoma). The remaining twenty-four cases (eight melanomas, fourteen basal cell carcinomas, one squamous carcinoma and one Bowen's disease) all showed patchy or complete loss of membrane β_2m staining (Table 1). Thus the melanoma and squamous cell samples showed constant HLA heavy chain but variable β_2m staining while the basal cell carcinomata consistently showed positive HLA heavy chain but negative β_2m staining in all cases.

DISCUSSION

This work demonstrates for the first time in epidermal tumours dissociation of expression of HLA heavy chain and β_2 microglobulin on the cell surface of twenty-four out of twenty-nine cutaneous malignancies and confirms previous studies concerning the loss of β_2m demonstrated by the immunoperoxidase technique using polyclonal antisera on paraffin-embedded malignant skin tumours (Tjernlund & Forsum, 1977; Turbitt & MacKie, 1981; Korthals *et al.*, 1981.

The use of frozen sections avoids possible changes in cell surface membrane antigens which may occur with formalin-fixed paraffin-embedded materials. Moreover, monoclonal antibodies minimize non-specific staining which can arise in the immunoperoxidase technique.

Persistence of HLA heavy chain with loss of β_2m from the cell surface could be explained in different ways. One possibility is that in malignant cells the β_2 molecule is no longer available to specific antibodies—the tri-dimensional structure is modified or the peptide chain is altered, although the HLA heavy chain is not; if this were the case, the antibodies could not react with the antigen.

An alternative explanation is that β_2m is not synthesized by the malignant cells and is completely absent from the cell surface as a consequence of changes either in the genetic material of the cells or in the synthetic pathways.

A third possibility is related to several reports that in patients with a wide variety of malignant tumours serum β_2m levels may be abnormally elevated as compared with those of age-matched controls (Kithier *et al.*, 1974; Shuster, Gold & Povlik, 1976). Tumour cells have been shown to produce large amounts of β_2m *in vitro*, suggesting that they might represent a major site of β_2m synthesis *in vivo* (Nilsson, Evrin & Welsh, 1974). Turnover studies of lymphocyte membrane-bound β_2m indicate that both β_2m and the heavy chain are produced at similar rates. In culture supernatant fluids, only free β_2m is detectable, indicating that during the normal turnover of HLA antigens β_2 is dissociated from the heavy chain and released in its free form in the extracellular medium (Cresswell *et al.*, 1974).

These experiments *in vitro* are compatible with studies showing that at least 95% of β_2 found in serum and urine is the free monomer. Very little is associated with other molecules including HLA (Vincent *et al.*, 1978). Despite the lack of direct evidence it is likely that β_2m present in serum and urine is derived from the turnover of cell membranes. The turnover and release of β_2m in cutaneous malignancies is therefore likely to be higher than in normal cells. Our results

FIGURE 1. HLA heavy chain staining of normal epidermis with diamino benzidine (DAB). Note strong membrane staining and weaker peripheral cytoplasmic staining, (DAB).

FIGURE 2. HLA heavy chain staining of basal cell papilloma. Note essentially similar picture to Fig. 1. In general the more deeply placed cells appear to stain more intensely, (DAB).

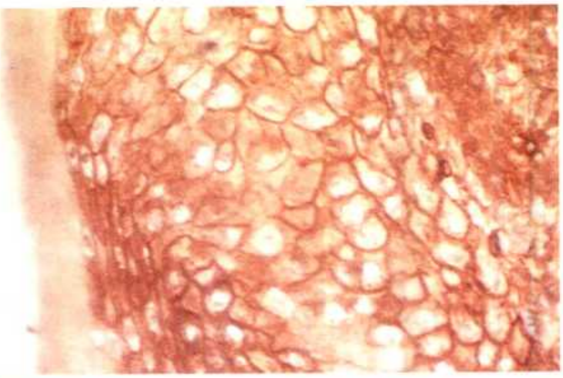
FIGURE 3. HLA heavy chain staining of basal cell carcinoma. Intense positive staining is seen, (antibody dilution 1:100, DAB).

FIGURE 4. Sequential section to Fig. 3 stained for β_2 microglobulin. Note absence of staining on basaloid cells and only patchy positivity of the vasculature, (antibody dilution 1.150, DAB).

FIGURE 5. Basal cell carcinoma stained for β_2 microglobulin Note again total absence of staining in the tumour, (DAB).

FIGURE 6. Subcutaneous deposit of malignant melanoma stained for HLA heavy chain with amino ethyl carbazole (AEC) using the biotin–avidin system. Note intense positivity of overlying epidermis and positive staining of the tumour cells.

FIGURE 7. Section of same tumour stained for β_2 microglobulin. Note absence of staining.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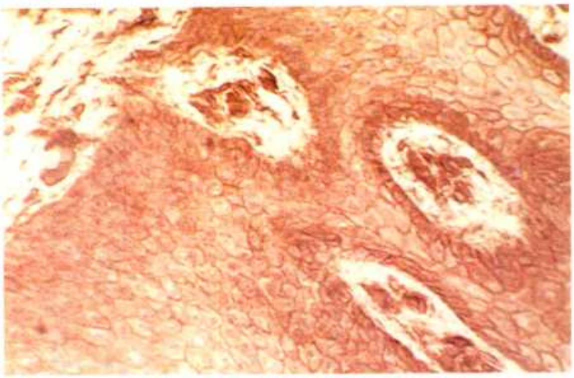
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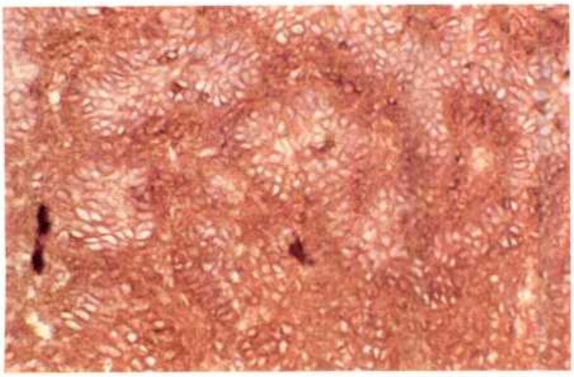
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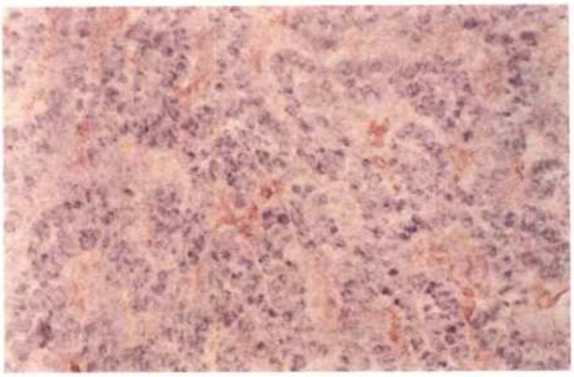
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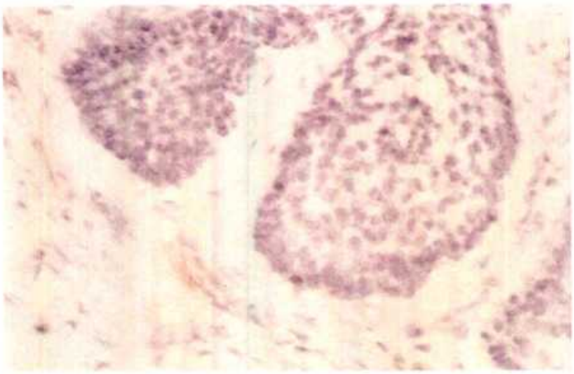
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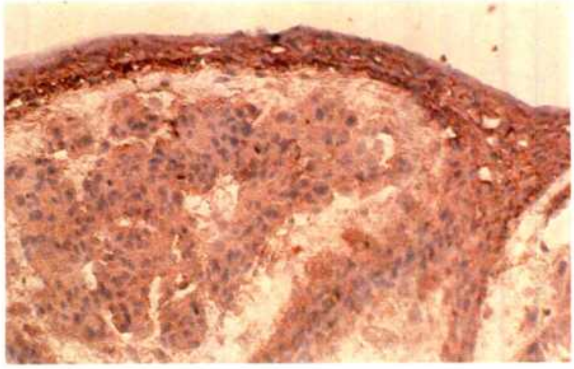
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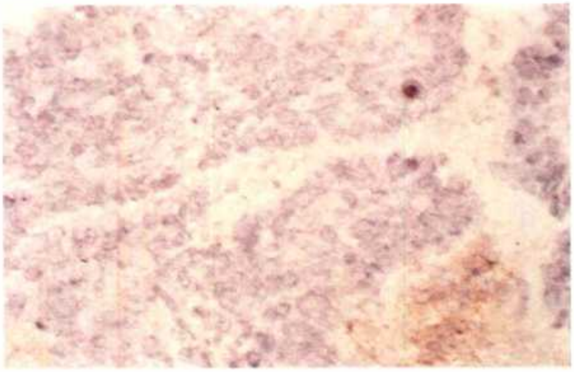
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may therefore by explained by the more rapid turnover and hence lower concentration of β_2m on the malignant cell surface.

This dissociation of expression of the HLA heavy and light chains on the surface membrane of cutaneous malignancies is of interest in that it may indicate a greater involvement of the genetic material of chromosome 15 in the neoplastic process than of that of chromosome 6. The patchy nature both of HLA heavy chain expression and of β_2 expression in those lesions in which it persists is further evidence of the heterogeneity of tumour populations indicated by other monoclonal antibody studies (Ashall *et al.*, 1982).

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Skin calcium-binding protein immunoreactivity in basal cell carcinoma

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SUMMARY

Skin calcium-binding protein (SCaBP) is a low molecular weight protein of unknown function which is localized in the cytoplasm of epidermal basal cells. We have examined SCaBP immunoreactivity in basal cell carcinoma (BCC) since BCC cells retain some of the characteristics of basal cells, including the capacity for proliferation and migration from the dermo-epidermal junction. We also examined immunoreactivity to basement membrane zone (BMZ) antigens (type IV collagen, laminin and bullous pemphigoid antigen) in BCC. We found that all BCC cells showed SCaBP immunoreactivity regardless of the histological type or position of the cells. BMZ antigens were expressed around tumourous buds, although bullous pemphigoid antigen may also present a cytoplasmic immunoreactivity. Our results suggest that SCaBP immunoreactivity is independent of the basal position of the cell, but may be related to the proliferative capacity and the undifferentiated state of the cell.

We have recently demonstrated the presence of a 'skin calcium-binding protein' (SCaBP) in the basal layer of the rat epidermis (Laouri *et al.*, 1980; Pavlovitch *et al.*, 1980; Saurat *et al.*, 1981a). This protein is localized in the cytoplasm of the basal cell (Saurat *et al.*, 1981a).

SCaBP has a low molecular weight (12,000 daltons), is acidic in nature and has a high content of phenylalanine (Rinaldi *et al.*, 1982). The amino acid sequence of SCaBP is very similar to that of parvalbumin, another calcium-binding protein. SCaBP differs however from parvalbumin with regard to its antigenic properties, the presence of numerous phenylalanine residues and its calcium affinity constant. Each molecule of SCaBP binds two calcium ions with high affinity (Rinaldi *et al.*, 1982). The function of SCaBP is unknown. Recently it has been shown that the pattern of growth and differentiation of mouse epidermal cells in culture can be shifted in favour of continued proliferation by reducing the calcium level of the medium to 0·05–0·1 mM. Thus extracellular calcium concentration regulates epidermal growth and differentiation in cell cultures (Hennings *et al.*, 1980; Yuspa *et al.*, 1982; Dykes, Jenner & Marks, 1982). The

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mechanism by which this regulation is achieved is unknown. We found that SCaBP was expressed in a high proportion of epidermal cells cultured in a low calcium concentration medium, whereas increasing calcium concentration sharply decreased the percentage of cells expressing SCaBP (Saurat *et al.*, 1981b; Didierjean *et al.*, 1983).

SCaBP is detectable in the basal cells of mouse embryo epidermis at a stage of embryonic development at which cells other than basal cells are capable of proliferation (Saurat *et al.*, 1981b). Therefore, the localization of SCaBP in the basal cell layer might be related more to the basal position of the keratinocyte (e.g. due to the adhesive process to the dermo-epidermal junction, etc. (Patel *et al.*, 1981) than to the proliferative capacity of this layer.

In order to investigate this issue, we have studied the localization of SCaBP within basal cell carcinoma since in this tumour, keratinocytes retaining basal cell characteristics, including their proliferative capacity (Weinstein & Frost, 1970; Heenen, Achten & Galand, 1973), migrate from the dermo-epidermal junction. Immunostaining of antigens of the dermo-epidermal junction such as bullous pemphigoid antigen, type IV collagen and laminin, was therefore included in the study. In addition, the mapping of SCaBP immunoreactivity in BCC is useful for workers interested in the calcium dependency of tumorigenic keratinocytes (Yuspa *et al.*, 1982).

METHODS

Antiserum

A rabbit serum containing antibodies against purified rat SCaBP was obtained as previously described (Laouri *et al.*, 1980; Saurat *et al.*, 1981a). Briefly, SCaBP from whole Sprangley albino rat skin was extracted by homogenization, and purified by chromatography. An antiserum to the purified protein was raised by immunization of white rabbits. Its specificity and cross reactivity were tested with Ouchterlony's double immunodiffusion technique, and polyacrylamide gel immunoelectrophoresis. Briefly, a single precipitation line was obtained with both skin extract and purified SCaBP with the former technique (Laouri *et al.*, 1980). With the latter, a unique precipitation peak was observed. This means that SCaBP antiserum is monospecific and directed against only the SCaBP protein (Rizk, 1983). This protein binds calcium as demonstrated by incubation with $^{45}Ca^{2+}$ (Rinaldi *et al.*, 1982). This antiserum has been shown to react specifically with the cytoplasm of the basal cells in the epidermis of several species including humans; a positive reaction was detected up to a 1:620 dilution (Saurat *et al.*, 1981a). In this study it was used at dilutions of 1:20, 1:100 and 1:500. Absorption procedures with purified SCaBP have been reported in detail previously (Saurat *et al.*, 1981a). Serum from a patient with bullous pemphigoid (antibody titre greater than 1:50) was used at a dilution of 1:10.

Rabbit antisera to laminin and type IV collagen were kindly provided by Prof. Fusenig, German Cancer Research Centre, Heidelberg, G.D.R. and used at dilutions of 1:10 and 1:100.

Indirect immunofluorescence (IIF)

Fifty normal human skin specimens, six basal cell carcinomas (BCC) and one squamous cell carcinoma (SCC) were obtained from excision specimens under local anaesthesia with 1% lignocaine. Half of the specimens were processed for routine histology. The other half were immediately frozen in liquid nitrogen and stored at −70 °C. They were serially sectioned with a cryostat microtome set at 4 μm. The tissue sections were incubated with the corresponding antiserum for 30 min at room temperature; after washing for 30 min in PBS, they were incubated with commercially prepared fluorescein isothiocyanate (FITC) labelled sheep anti-rabbit IgG antiserum (Cappel Laboratories, Downing PA, U.S.A.; specific antibody concentration 8·0 mg/ml, F/P ratio 3·43 mg/g; working dilution in PBS 1:160 to obtain 50 mg/ml of specific antibodies) for SCaBP, laminin and type IV collagen, or with commercially prepared FITC labelled goat anti-human IgG antiserum (Hyland, Deerfield, Illinois, U.S.A.; specific antibody concentration 1·8 mg/ml, F/P molar ratio 2·2; working dilution in PBS 1:36 to obtain 50 mg/ml of specific antibodies) for bullous pemphigoid antigen.

The slides were then washed for 30 min in PBS and mounted in Fluorep® (sodium azide; BioMérieux Laboratories). They were examined with a Zeiss fluorescent microscope equipped for incident illumination and photographed with Kodak 400 ASA film.

RESULTS

Normal human epidermis

The anti-SCaBP serum was found to give a bright staining of the epidermal basal cell layer as previously described (Saurat *et al.*, 1981a) (Table 1 and Fig. 1). Laminin, type IV collagen and BP antigen were detected in a linear pattern at the dermo-epidermal junction and laminin and collagen type IV around blood vessels.

TABLE 1. Immunoreactivity of basal cell carcinoma (BCC)

							Basal cell carcinomas					
	Normal epidermis			Overlying and adjacent normal epidermis			Buds			Vicinity of peritumoral lacunae		
No.	1:20*	1:100	1:500	1:20	1:100	1:500	1:20	1:100	1:500	1:100		
1	4+**	3+	2+	4+	3+	2+	3+ to 4+	2+ to 3+	+ to 2+	N	N	N
2	4+	3+	2+	4+	3+	2+	4+	3+	2+	4+	3+	2+
3	4+	3+	2+	4+	3+	2+	2+ to 3+	+ to 2+	+	3+	2+	+
4	4+	3+	2+	4+	3+	2+	3+	+ to 2+	+	3+	+ to 2+	+
5	4+	3+	2+	4+	3+	2+	2+	+ to 2+	+	N	N	N
6	4+	3+	2+	4+	3+	2+	4+	3+	+ to 2+	4+	3+	+ to 2+

* SCaBP antiserum dilution.

** SCaBP immunoreactivity is expressed from 4+ to (−) in comparison to the SCaBP immunoreactivity of the normal human epidermis reacted with SCaBP antiserum diluted 1:20. (quoted 4+) N = No lacuna detected.

Basal cell carcinoma

Standard histology showed that the carcinomas were either of superficial multicentric type, adenoid type, solid type, or shared many of these features. No histological evidence of keratinizing differentiation was found.

SCaBP immunoreactivity was found in all the cells of BCC irrespective of their histological types. The pattern was consistent with a cytoplasmic staining (Figs 2 and 3) and there was no nuclear staining. The immunoreactivity was usually as bright as in normal adjacent skin (Fig. 2) and normal distant skin (Table 1). There were, however, some differences in the intensity of the SCaBP immunoreactivity within BCC cells in the same bud. In some areas immunoreactivity of peripheral BCC cell was lighter than the central ones. In other areas, the contrary could be observed, while an even more irregular staining pattern was observed in some other buds. At any

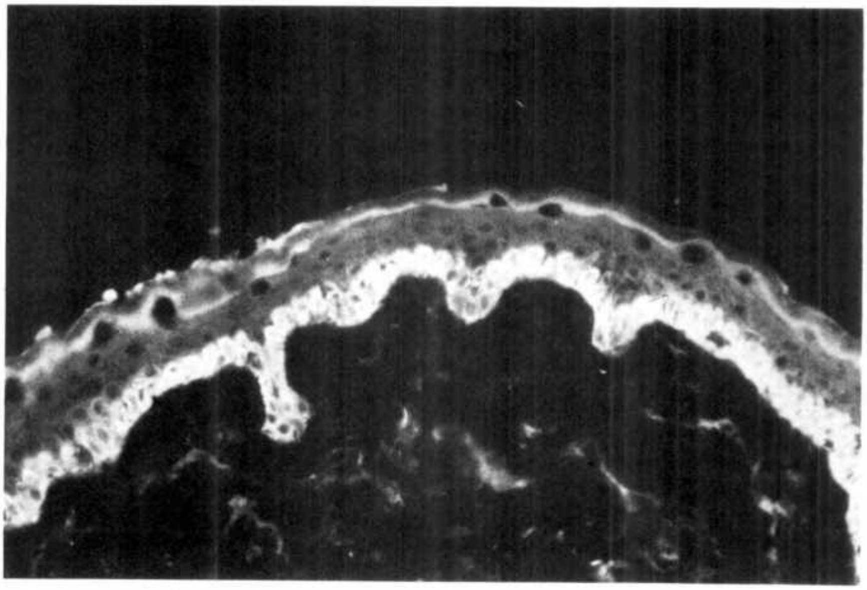

FIGURE 1. Normal human skin: SCaBP immunoreactivity in basal cell layer (× 98).

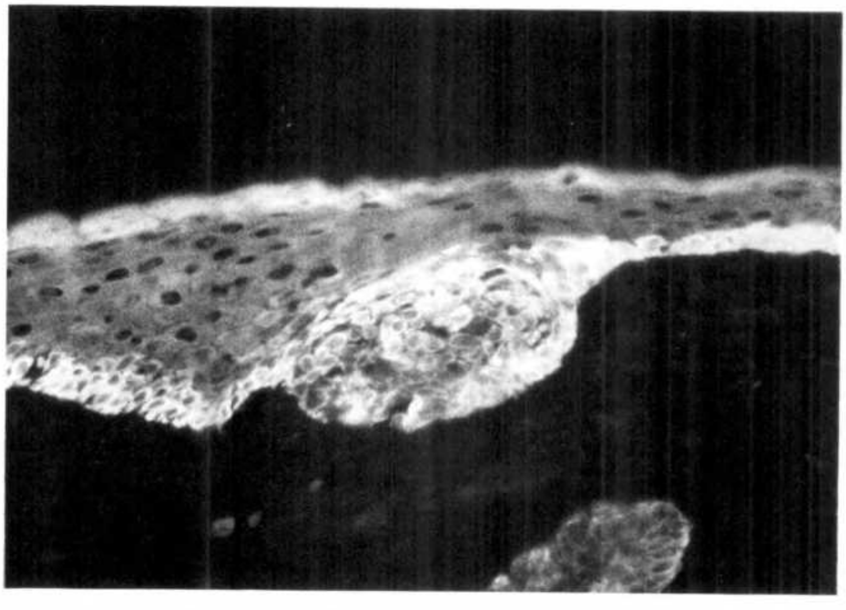

FIGURE 2. Superficial multicentric BCC. The SCaBP immunoreactivity of the carcinomatous buds was as bright as in adjacent basal cells (× 98).

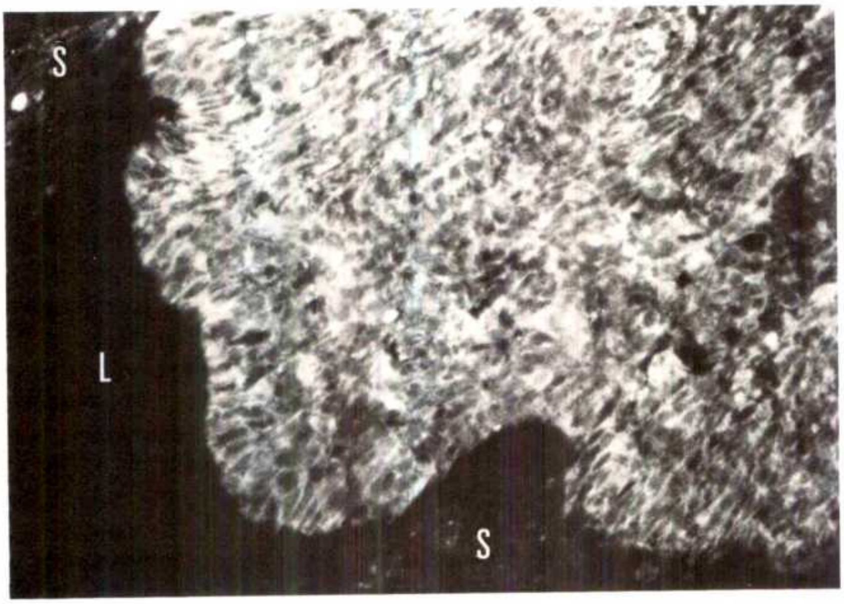

FIGURE 3. Solid type of BCC. All the carcinomatous cells were SCaBP immunoreactive. SCaBP immunoreactivity remained unchanged in carcinomatous cells adjacent to a lacuna. L = lacuna. S = stroma. (× 98.)

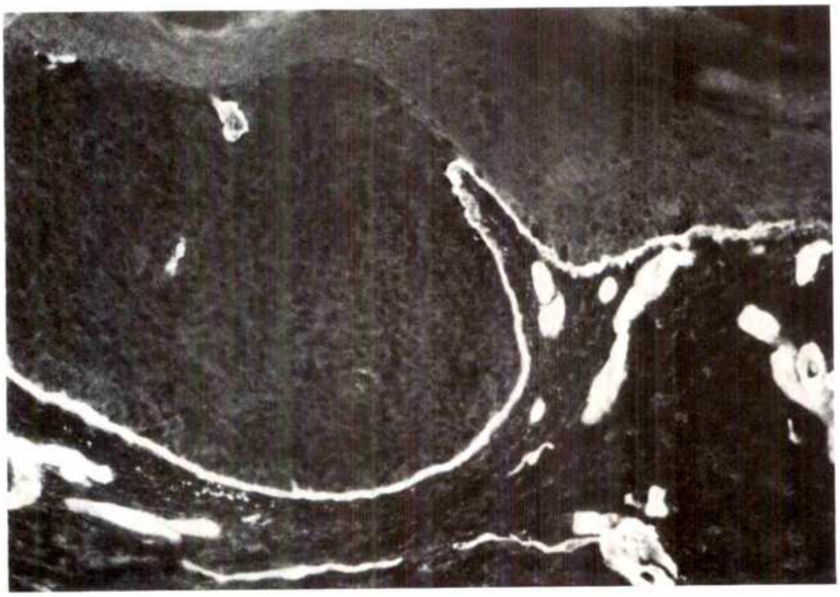

FIGURE 4. Laminin immunostaining around the BCC buds (× 65).

rate, SCaBP immunoreactivity was present in all BCC cells and no loss of SCaBP immunoreactivity was detectable in cells in the centre of the bud. When peritumoral lacunae occurred, SCaBP immunoreactivity remained unchanged in the cells adjacent to the lacuna (Fig. 3).

In all BCC, laminin and type IV collagen were detected around the tumours (Fig. 4). There was no staining within the carcinomatous buds. The BP immunoreactivity was more variable, with a linear continuous or discontinuous pattern surrounding the carcinomatous nests, or sometimes no staining at all. Moreover some of the BCC cells showed a cytoplasmic staining pattern. This cytoplasmic BP Ag immunoreactivity could be seen either in peripheral cells which have close contacts with the basement membrane or in a central position. This pattern correlated neither with a particular histological type of BCC nor with any distinctive alteration in SCaBP immunoreactivity. In a given specimen only a few buds showed this cytoplasmic BP immunoreactivity.

Normal epidermis adjacent to or overlying the BCC

Immunoreactivity of the four antigens studied was identical in the epidermis adjacent to and overlying the BCC, as in normal epidermis. When the BCC buds were attached to the undersurface of epidermis, there was no discontinuity of the staining pattern between normal epidermis and the carcinomatous bud (Fig. 2). The overlying epidermis always showed a SCaBP immunoreactivity similar to normal epidermis, even when compressed or displaced by the tumourous bud.

Squamous cell carcinoma

The SCC examined was well differentiated and only a few cells showed SCaBP immunoreactivity. These cells were usually confined to the periphery of the tumorous proliferation (Fig. 5). In

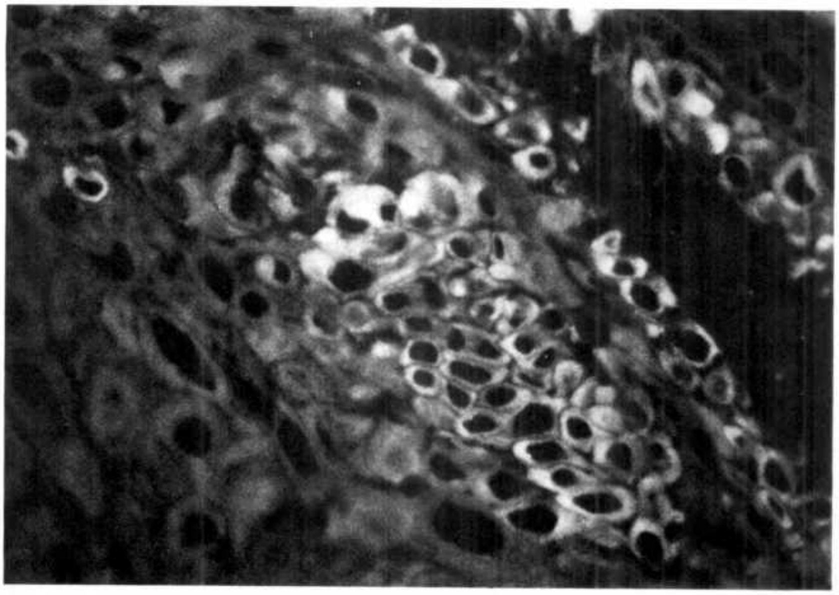

FIGURE 5. Squamous cell carcinoma. SCaBP immunoreactivity is expressed only in the peripheral cells of the carcinoma (× 165).

the one to three cellular layers, only a few scattered cells were positive for SCaBP immunoreactivity in the central areas of these buds, with clearly negative cells around them. Laminin, type IV collagen and BP Ag were detected at the carcinoma-stroma junction and they showed a linear pattern. We observed neither a staining discontinuity with BP Ag nor cytoplasmic immunoreactivity.

Controls

Neither the FITC labelled sheep anti-rabbit IgG antiserum nor the FITC labelled goat anti-human IgG antiserum reacted with normal skin or tumorous material. Previous absorption of SCaBP antiserum with purified SCaBP abolished the staining as detailed previously (Saurat *et al.*, 1981a). No staining was observed with pre-immune serum.

DISCUSSION

SCaBP immunoreactivity was found in BCC with similar intensity to that found in normal human skin and the adjacent overlying epidermis. It was present in the superficial buds, as well as in the more deeply situated tumours and the staining pattern was consistent with a cytoplasmic distribution, as seen in normal basal keratinocytes. Even if the intensity of the immunoreactivity was not perfectly identical within all the buds, it can be assumed that, by and large, BCC do express SCaBP immunoreactivity in all the cells. This is in contrast to the immunoreactivity pattern observed in SCC where only the periphery of the buds was positive.

The BMZ antigens laminin and type IV collagen were found only in the periphery of the tumours, as previously reported (Stanley *et al.*, 1982a; Weber *et al.*, 1982; Mérot, Didierjean & Saurat, 1982).

The expression of BP Ag in some cells within the carcinomatous buds may be the result of a defective secretory function of BCC cells (Stanley *et al.*, 1981; Stanley *et al.*, 1982b; Woodley *et al.*, 1980).

Therefore SCaBP positive cells can be found which are not in close contact with basal membrane zone proteins; this observation suggests that the basal position of the cell is not *per se* a prerequisite for the expression of SCaBP.

Since BCC cells can be considered as proliferative (Weinstein & Frost, 1970; Heenen *et al.*, 1973) and not differentiated keratinocytes (Zelickson, 1962; Lever & Schaumberg-Lever, 1975) the present findings support the hypothesis that the basal cell localization of SCaBP within normal epidermis is more directly related to these two characteristics than to adhesion to the basal membrane zone. The persistence of SCaBP immunoreactivity in the suprabasal cells of BCC (in contrast to SCC and suprabasal cells in normal epidermis) correlates well with the absence of keratinized differentiation in the BCC we studied. In this respect SCaBP immunoreactivity has a distribution pattern completely opposite to involucrine, the substrate of the calcium-dependent transglutaminase (Rice & Green, 1979). It would be interesting to know the distribution of involucrine in BCC. Taken together, these observations strongly suggest that SCaBP is a protein expressed in keratinocytes which have not entered the process of terminal differentiation.

The persistence of SCaBP in BCC must also be considered from an oncological point of view. It is well established that carcinomatous cells can express calcium-binding proteins. Increased levels of calmodulin, the ubiquitous modulator calcium-binding protein, have been found in several types of mouse, rat and human tumours (Macmanus *et al.*, 1982). The seemingly *de novo* appearance of another calcium-binding protein named oncomodulin was also observed in the same tumour cells, whereas this protein was not detectable in normal tissues.

Oncomodulin is a 11,500 daltons acidic calcium-binding protein which has been shown to be distinct from SCaBP (Rinaldi *et al.*, 1982). Oncomodulin is regarded as a tumour-specific modulator that will intrude on calmodulin-dependent steps during the initiation of DNA synthesis in tumour cells (Macmanus *et al.*, 1982). Oncomodulin has not been detected to our knowledge either in normal or in malignant keratinocytes. In this respect it should be emphasized that normal keratinocytes respond *in vitro* to extracellular calcium concentration in the opposite way to other normal cells. Unlike keratinocytes, many other culture systems require a calcium level of the order of 1 mM for optimum growth, whereas concentrations below 0·1 M which are stimulatory for keratinocytes, have been reported as inhibitory for other normal cells (Boynton *et al.*, 1974, 1977). MacManus *et al.* (1982) have shown that oncomodulin was able to re-initiate DNA synthesis in isolated liver cells which were arrested near the G_1/S boundary of the cell cycle by decreasing the calcium in the medium from 1·5 to 0·02 mM; therefore, by enabling these non-tumorogenic cells to make DNA in a low-calcium medium, oncomodulin had endowed them with a common property of neoplastic liver cells. These observations point to the importance of Ca^{2+} binding in the control of cell proliferation.

As far as keratinocytes are concerned, we have observed that a high percentage of normal keratinocytes grown *in vitro* in a low calcium concentration expressed SCaBP immunoreactivity whereas a dramatic decrease in SCaBP-positive cells was observed when these cells were incubated with a high calcium concentration (Didierjean *et al.*, 1983). Such experiments should be done on tumorigenic keratinocytes since it has been shown that *in vitro* transformed keratinocytes do not respond to increased extracellular calcium, which induces terminal differentiation in normal keratinocytes (Yuspa *et al.*, 1982). This would determine whether the absence of response to high extracellular concentration is associated with the persistence of SCaBP. In this respect, our present observation of the expression of SCaBP immunoreactivity within all the BCC cells may be an important *in vivo* finding.

ACKNOWLEDGMENTS

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The distribution of keratin type intermediate-sized filaments in so-called mixed tumour of the skin

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SUMMARY

Epithelial cells can be distinguished from various non-epithelial cells by the presence of keratin-type intermediate-sized filaments, which can be detected by immunofluorescence microscopy, using antibodies to α-keratin. In the present study, two types of antibodies were obtained. One of them was specific for α-keratin (mol. wt. 49,000 to 69,000 daltons) in whole epidermis, and the other for α-keratin (mol. wt. 62,000 and 69,000 daltons) in prickle and granular cells but not in basal cells. Four cases of so-called mixed tumour of the skin were studied by immunofluorescence microscopy using these antibodies. Tumour cell nests of cuboidal and polygonal cells, tubular structures and keratinous cysts reacted with these antibodies, as did individually-dispersed tumour cells within the myxoid and chondroid matrix. These results indicate that all the tumour cells of the so-called mixed tumour of the skin are of epithelial origin. Differences in staining intensity between these tumour cells and their specificity for these two antibodies are discussed with reference to keratin differentiation in tumour cells.

Various cells of vertebrate tissues contain three fibrous skeletal elements, i.e. microtubules (20–25 nm), microfilaments (6 nm) and intermediate-sized filaments (7–11 nm). There are several types of intermediate-sized filaments such as cytokeratin filaments in epithelial cells, desmin filaments in mesenchymal cells, neurofilaments in neurons and glial fibrillar acidic protein-containing filaments occurring in astrocytes (Franke *et al.*, 1981). Recently antibodies to different types of intermediate-sized filaments have been used to distinguish cells which contain these filaments (Franke *et al.*, 1978; Franke *et al.*, 1980; Bannasch *et al.*, 1980; Viac *et al.*, 1980; Summerhayes *et al.*, 1981). Epithelial cells of diverse organs can be identified and distinguished from various non-epithelial tissues by the presence of intermediate-sized

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filaments (Franke *et al.*, 1979). These proteins have been shown to be similar to epidermal prekeratin by immunofluorescence microscopy, using antibodies to prekeratin. They proposed the use of this specific structure, α-keratin, as a criterion for true epithelial character or origin.

So-called mixed tumour of the skin was first described by Nasse (1892). Controversy over the origin of the tumour has continued until the present time, because of the existence of myxoid and chondroid areas (Morehead, 1945; Halpert & Hackney, 1950; Halpert & Brelsford, 1954; Hernandez, 1976). In this report, cells of so-called mixed tumour of the skin were examined by immunofluorescence microscopy using antibodies to α-keratin in order to identify and distinguish the origin of these cells and to observe the distribution of keratin type intermediate-sized filaments.

METHODS

Four different specimens of so-called mixed tumour of the skin were surgically removed from the forehead of a 51-year-old woman, the upper lip of a 38-year-old woman, the ala nasi of a 29-year-old woman and the upper lip of a 25-year-old man. The tissues were immersed in 10% formalin, and after approximately 24–48 h they were treated with xylol and embedded in paraffin. Sections 4 μm in thickness were cut on a microtome, transferred to glass slides and dried overnight at 45°C. They were subsequently deparaffinized with xylol and an alcohol series. They were then examined by immunocytochemical procedures. Normal human skin was used as control material.

Preparation of α-keratin polypeptides

Two α-keratins were extracted separately from the stratum corneum of callus of normal volunteers, and the non-lesional sole of a psoriatic patient respectively, using a modification of the technique of Steinert, Peck & Idler (1980). The specimens were minced into fine pieces, homogenized and washed thoroughly with several changes of phosphate-buffered saline (PBS) until the absorbance of the last supernatant was less than 0·05 at 280 nm. The precipitates were then extracted in a buffer of 8 M urea, 0 05 M Tris-HCl (pH 9·0), 50 mM 2-mercaptoethanol for 3 h at 37°C. Cellular debris was removed by centrifugation at 40,000 × ***g*** for 30 min. The extracts were dialysed against 1,000 vol. of 5 mM Tris-HCl (pH 8·0) containing 50 mM 2-mercaptoethanol, and the dialysates were mixed with 1·0 M sodium acetate (pH 5·5). By subsequent centrifugation at 40,000 × ***g*** for 1 h, the pellets were obtained, which were rinsed with PBS and collected by centrifugation at 40,000 × ***g*** for 1 h. The precipitates were subjected twice more to the same purification procedures and purified α-keratins were obtained. Purified α-keratins were desalted by dialysis and were lyophilized.

Disc SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

Portions of 40–60 μl of solution, which contained 20–30 μg of protein, were analysed on 5·6% polyacrylamide gel (8 cm long × 0·7 cm thick) containing 0·1% SDS, prepared by the method of Fairbanks & Wallach (1971). The molecular weight of the polypeptides were estimated using bovine serum albumin (mol. wt. 67,000), egg albumin (mol. wt. 45,000), carbonic anhydrase (mol. wt. 30,000), chymotrypsinogen A (mol. wt. 25,000), trypsin inhibitor (mol. wt. 20,000) and cytochrome C (mol. wt. 12,500) as standards.

Electron microscopy

α-Keratins were repolymerized ***in vitro*** and examined by electron microscopy after negative staining with 2% phosphotungstic acid at pH 6·8.

Antibody production

α-Keratins (2 mg/ml) in 8 M urea containing 1 mM dithiothreitol and 10 mM Tris-HCl buffer (pH 9·0) were mixed with an equal volume of Freund's complete adjuvant and injected intradermally into multiple sites in Japanese white rabbits. The animals were boosted weekly, 3 weeks after the first injection. The course of antibody production was followed in the weekly bleedings using a modified Ouchterlony double diffusion test and indirect immunofluorescence test. Antibodies were purified by salting out using ammonium sulphate.

Ouchterlony double diffusion test

α-Keratins were dissolved (2 mg/ml) in 8 M urea, 0·1 M Tris-HCl, pH 8·3, containing 1 mM DTT and approximately 10–15 μl placed in the centre well, while 10–15 μl of twofold serial dilutions of antiserum and pre-immune serum were placed in the outer wells.

Immunocytochemical procedures

Antibodies to α-keratins were applied to deparaffinized sections on the glass slides for 45 min at room temperature. After washing well with PBS, commercially available (Medical and Biological Laboratories LTD, Tokyo) fluorescein-labelled goat anti-rabbit IgG (F/P = 1·7) was added and the samples were mounted in glycerine. Specimens were observed using a Olympus fluorescence microscope (Olympus, Tokyo, Japan). Immunological specificity was tested by blocking the reaction with non-labelled goat anti-rabbit IgG, and pre-immune rabbit serum was used instead of the primary antiserum as a control. Semi-quantitative analysis of the intensity of immunofluorescence was carried out. The intensity of immunofluorescence of tumour cells was compared with that of the prickle cells of normal epidermis within the same section in which tumour cells were contained. Intensity similar to that of normal prickle cells was graded as three plus (+ + +), the less intense fluorescence was graded two plus (+ +) and the least intense fluorescence was one plus (+). A negative result was marked as minus (−).

Haematoxylin and eosin specimens

Haematoxylin and eosin specimens were prepared by using the same sections which were used for immunofluorescence micrography after immunofluorescence micrographs were taken.

RESULTS

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) of α-keratin polypeptides from stratum corneum of human callus revealed six polypeptides with molecular weights of 49,000 to 69,000 daltons and this α-keratin specimen was designated as α-keratin A in the present study. SDS-PAGE of α-keratin polypeptide from stratum corneum of non-lesional sole of a psoriatic patient showed two polypeptides with molecular weights of 62,000 and 69,000 daltons and this α-keratin specimen was designated as α-keratin B. These SDS-PAGE patterns are shown in Fig. 1.

α-Keratin A and B were repolymerized and on electron microscopy exhibited the fibrous structure (not shown here).

Antisera to α-keratin A and B produced in Japanese white rabbits were tested by the Ouchterlony technique. Two precipitin lines were observed between α-keratin and antiserum, showing the specific activity of the serum against α-keratin (Fig. 2).

Antibody to α-keratin A strongly stained all living cell layers of normal human epidermis. On

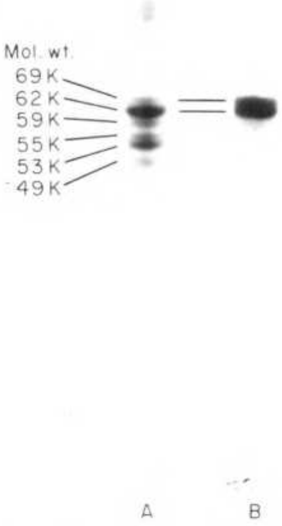

FIGURE 1. SDS-polyacrylamide gel electrophoretic patterns of α-keratins extracted from callus of normal human (A) and from stratum corneum non-lesional sole skin of a psoriatic patient (B).

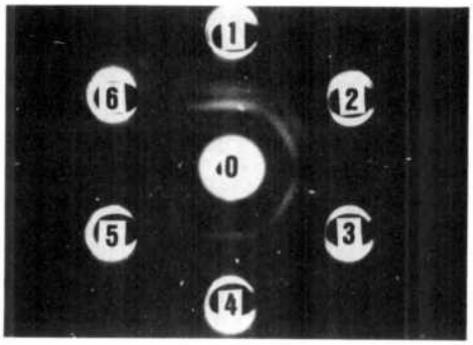

FIGURE 2. Ouchterlony double diffusion test of α-keratin. α-Keratins were dissolved (2 mg/ml) in 8 M urea, 0·1 M Tris-HCl, pH 8·3, containing 1 mM DTT, and approximately 10 to 15 μl of the solution was placed in the centre well (0), while 10 to 15 μl of the twofold serial dilution of antiserum (1–5) and pre-immune serum (6) were placed in the outer wells. Two precipitin lines were observed between α-keratin and antiserum.

the other hand, antibody to α-keratin B stained only suprabasal cells of normal human epidermis. However, both antibodies failed to stain horny cell layers (Fig. 3).

So-called mixed tumour of the skin

The tumours in the present cases consisted of nests of cuboidal or polygonal cells, cellular nests forming tubular structures, myxoid areas, chondroid areas and keratinous cysts as seen in previously reported typical cases. Prickle cells of normal epidermis on the same biopsy specimen

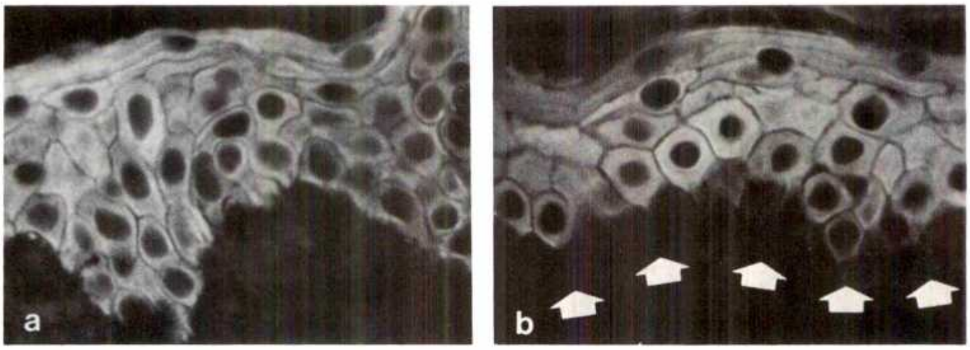

FIGURE 3. Sections of formalin-fixed and paraffin-embedded tissues of normal human skin stained with rabbit antibodies to α-keratins and with FITC-labelled anti-rabbit goat serum. Antibody to α-keratin A strongly stained all living cells including basal cells (a), while antibody to α-keratin B stained only suprabasal cells of normal human epidermis (b). ↑:Negative basal cell layer.

were used as a control for the standard intensity of fluorescence. Each tumour cell of the specimens was compared with this standard and graded for intensity of immunofluorescence.

Nests of cuboidal or polygonal cells in all the cases studied reacted with antibodies to α-keratin A and B, and showed an intense or moderate staining in the cytoplasm (Fig. 4). The tubular structures in the tumours reacted moderately with both antibodies (Fig. 5).

Dispersed cells within the myxoid matrix revealed a moderate to weak reaction around the nucleus with both antibodies (Fig. 6).

Tumour cells associated with chondroid matrix were seen in two of the four cases. These cells exhibited a weak staining with both antibodies (Fig. 7).

Tumour cells forming keratinous cysts, which were seen in two of the four cases, were stained strongly with both antibodies (Fig. 8). These immunological reactions were blocked with non-labelled goat anti-rabbit IgG, and these cells were not stained by pre-immune rabbit serum.

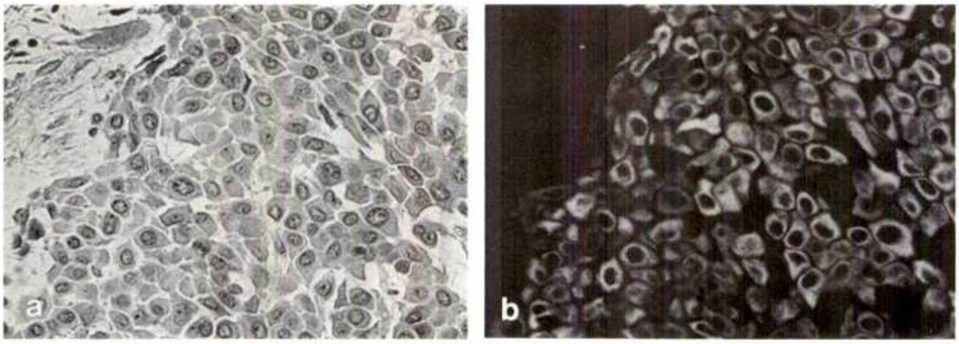

FIGURE 4. Section of formalin-fixed and paraffin-embedded tissue of so-called mixed tumour of the skin stained with haematoxylin and eosin (a), and antibody to α-keratin A (b). Both Figs 4a and 4b show the same portion of the same section, which was first stained with antibody and then with haematoxylin and eosin. Nests of cuboidal and polygonal cells were stained intensely with antibody to α-keratin A.

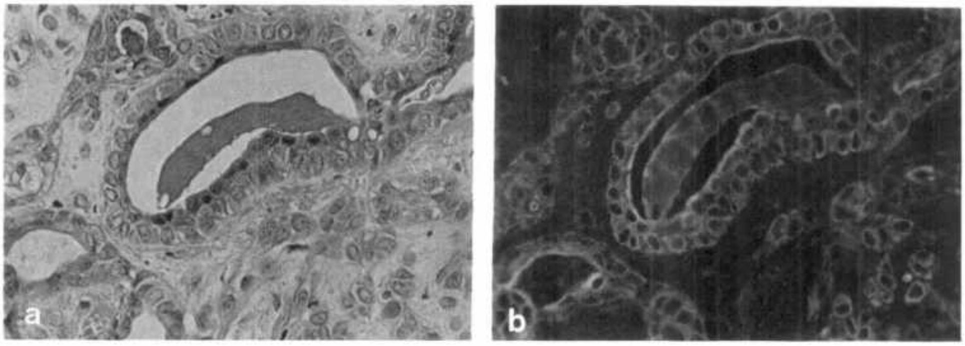

FIGURE 5. Section of tubular structure of the so-called mixed tumour of the skin stained with haematoxylin and eosin (a), and antibody to α-keratin A (b). Both Figs 5a and 5b show the same portion of the same section, which was first stained with antibody and then with haematoxylin and eosin. Tumour cells were moderately stained with antibody to α-keratin A.

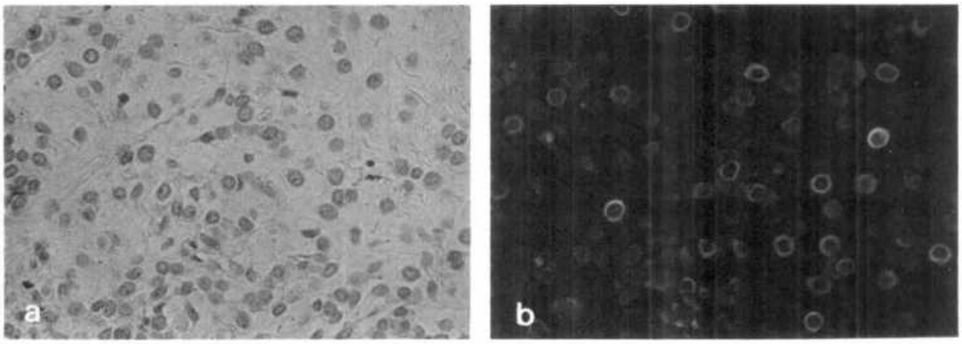

FIGURE 6. Section of myxoid area in the so-called mixed tumour of the skin stained with haematoxylin and eosin (a), and antibody to α-keratin A (b). Both Figs 6a and 6b show the same portion of the same section, which was first stained with antibody and then with haematoxylin and eosin. The cells dispersed within myxoid matrix revealed a moderate to weak staining with the antibody.

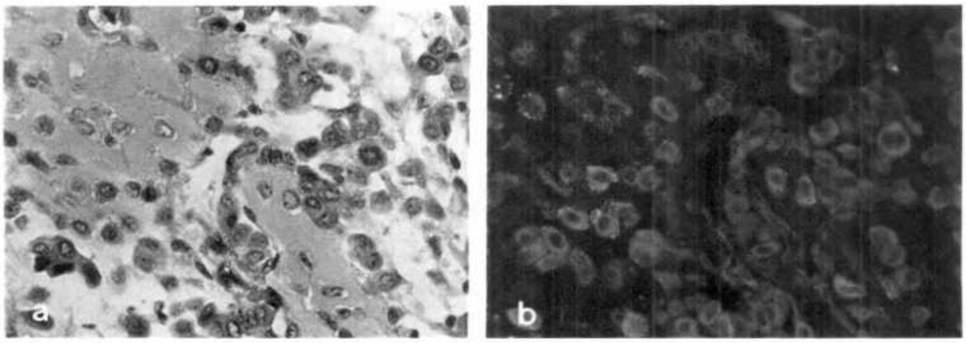

FIGURE 7. Section of chondroid area in the so-called mixed tumour of the skin stained with haematoxylin and eosin (a), and antibody to α-keratin A (b). Both Figs 7a and 7b show the same portion of the same section, which was first stained with antibody and then with haematoxylin and eosin. The tumour cells associated with chondroid matrix were stained weakly with the antibody.

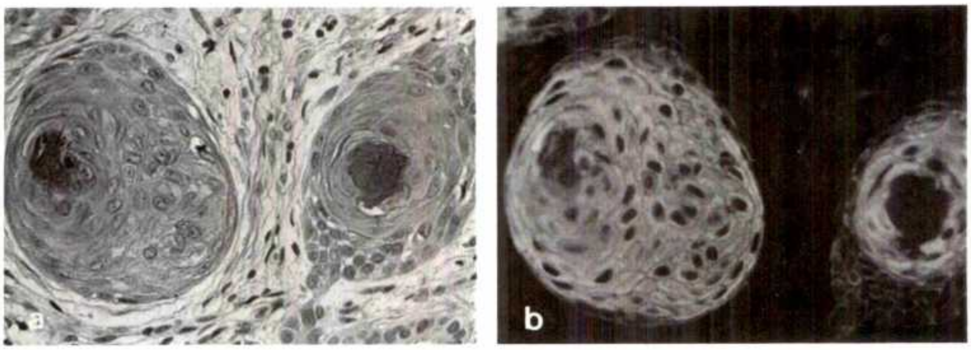

FIGURE 8. Section of keratinous cysts in the so-called mixed tumour of the skin stained with haematoxylin and eosin (a), and antibody to α-keratin A (b). Both Figs 8a and 8b show the same portion of the same section, which was first stained with antibody and then with haematoxylin and eosin. Tumour cells were stained strongly with the antibody.

TABLE 1. Staining properties of cells of so-called mixed tumours of the skin by immunofluorescence microscopy using antibodies to α-keratin

Case	Age/Sex Location		Nest	Tubular	Myxoid	Chondroid	Keratinous cyst
1.	51/F	Anti-A	+ + + ~ + +	+ +	+ + ~ +	+	+ + +
	forehead	Anti-B	+ + + ~ + +	+ +	+ + ~ +	+	+ + +
2.	38/F	Anti-A	+ + + ~ + +	+ +	+ + ~ +	+	/
	upper lip	Anti-B	+ + + ~ + +	+ +	+ + ~ +	+	/
3.	29/F	Anti-A	+ + + ~ + +	+ +	+ + ~ +	/	/
	ala nasi	Anti-B	+ + + ~ + +	+ +	+ + ~ +	/	/
4.	25/M	Anti-A	+ + + ~ + +	+ +	+ + ~ +	/	+ + +
	upper lip	Anti-B	+ + + ~ + +	+ +	+ + ~ +	/	+ + +

+ : Weakly stained.
+ + : Moderately stained.
+ + + : Strongly stained.
/ : Structural components which were not found in the section.

DISCUSSION

Nasse (1892) described a skin tumour resembling the mixed tumour of salivary glands. Controversy concerning the histogenesis of this tumour has continued to the present time. It is now generally accepted that the tumour is a neoplasm of epithelial origin (Headington & Mich, 1961; Hirsch & Helwig, 1961; Montgomery, 1967; Lever, 1975) but it contains myxoid and chondroid portions which appear to be non-epithelial with respect to their histological features. In our study all the cells in so-called mixed tumour of the skin, including cells within the myxoid and chondroid matrix, reacted with antibodies to α-keratin. This result suggests that all the cells composing the tumour were of epithelial origin, and that even the cells dispersed individually within the myxoid and chondroid matrix contained keratin-type intermediate-sized filaments. In considering the intensity of the immunological reaction with antibodies to α-keratin, we should consider two factors, namely the quantity and the quality of the α-keratin. We found some differences in intensity between tumour cells of different histological types in so-called mixed tumour of the skin (summarized in Table 1). Each histological structure in the tumour revealed almost the same staining intensity with both antibodies, although there were some differences between the different histological types. This result suggests that there may be quantitative differences in α-keratin amongst the different histological types of so-called mixed tumour of the skin.

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Distribution patterns of cytoplasmic microtubules in epidermal keratinocytes

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SUMMARY

The distribution of cytoplasmic microtubules in cultured guinea-pig keratinocytes was investigated using immunofluorescence (IF) microscopy with monospecific anti-tubulin antibodies and electron microscopy (EM). In culture, adherent cells displayed networks of thin fluorescent fibres, while a homogeneous and/or granular cytoplasmic IF was shown in the cells of upper layers as well as in trypsinized cells. By EM many microtubules were shown in adherent cells but there were fewer or none in the upper layers. An increase in calcium ion (Ca^{2+}) concentration and the addition of an ionophore (X537A) to the culture medium caused disassembly of microtubules. This effect was cancelled by a calmodulin inhibitor. Cryostat sections of normal human and guinea-pig epidermis stained with anti-tubulin antibodies showed a homogeneous and/or granular cytoplasmic IF from basal to granular layers but no detectable IF was seen in the horny layer. These results suggest that keratinocytes contain a cellular pool of tubulin in various states of polymerization and that microtubule disassembly may occur during differentiation, probably being regulated by Ca^{2+}–calmodulin complexes.

Cytoplasmic microtubules (MT) are one of the fibrous systems in all eukaryotic cells. MT consist primarily of polymerized chains of tubulin and each shows a straight and tubular ultrastructure with an outer diameter of approximately 25 nm. The major role of MT is formation and maintenance of cell shape; they also participate in cellular movement, secretion and transport of intracellular components and surface movement of membrane macromolecules (Raff, 1979). Several studies have raised the possibility that the relative distribution of a cellular pool of tubulin between polymerized and depolymerized forms may represent an important regulatory site for these MT-dependent functions, as reviewed by Raff (1979).

Using immunofluorescence (IF) microscopy with anti-tubulin antibodies, MT can be visualized as a radial distribution in several monolayer-cultured cell lines (Brinkley *et al.*, 1980).

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In the epidermis the presence of MT *in situ* has been shown by electron microscopy (EM) (Rupec, 1974). In this study, we have attempted to demonstrate the basic arrangement of MT in different layers of cultured keratinocytes and in cryostat sections of epidermis employing IF and EM techniques.

METHODS

Preparation of tubulin

Tubulin was purified from rat brains by the method of Shelanski, Gaskin & Cantor (1973). Brains were homogenized in the assembly buffer (pH 6·4) containing 0·1 M 2-(*N*-morpholino)-ethane sulphonic acid (MES), 1 mM ethyleneglycol-bis-(β-aminoethyl ether)-*N*,*N*′-tetraacetic acid (EGTA), 1 mM guanosine 5′-triphosphate (Sigma) and 0·5 mM magnesium chloride at 4°C. Assembly buffer-soluble proteins were allowed to polymerize in 4 M glycerol buffer at 37°C and then polymerized tubulin was again dissociated by cooling on ice. After repeating three cycles of the procedure, final pellets containing MT were obtained.

By a negative staining technique *in vitro*-assembled MT showed straight or slightly curved tubular ultrastructures with an outer diameter of approximately 25 nm. Sodium dodecyl sulphate–polyacrylamide gel electrophoresis of the preparation yielded a single band which corresponded to tubulin molecules with no visible contamination by other proteins.

Production of monospecific anti-tubulin antibodies

Antisera against tubulin were obtained from albino female rabbits immunized twice by footpad injections of a mixture of 5 ml of purified tubulin (total 1·2 mg, determined by the method of Lowry *et al.* (1951) and an equal volume of Freund's complete adjuvant (Difco) at a 1 month interval. Sera were collected 2 weeks after the last immunization; they were heat-inactivated and stored at −80°C until use (titre = 1:160, determined by indirect IF microscopy). The IgG fraction eluted out through a DEAE cellulose column was applied to tubulin-coupled, cyanogen bromide-activated Sepharose 4B (Pharmacia) affinity chromatography to obtain monospecific antibodies (Brinkley *et al.*, 1980). On immunodiffusion plates antibodies made a single major precipitin line with the purified tubulin preparation.

Cell culture

Primary cultures of guinea-pig keratinocytes were used in this study. Isolated keratinocytes were obtained from enzymatic digestion of sheets of back skin epidermis (Hartley strain) by 0·25% trypsin solution (1:250, Difco) at 37°C (Takigawa & Imamura, 1977) and suspended in Eagle's minimal essential medium (MEM) (approximate calcium ion level = 1·2 mM) containing 100 IU/ml of penicillin G, 100 μg/ml of streptomycin and 20% fetal calf serum. One millilitre of the suspension (5×10^5 cells) was seeded on a 35 × 10 mm plastic culture dish (Falcon) containing sterile glass coverslips and dishes were incubated in a moist chamber with 5% CO_2 and 95% air at 37°C. Between 7 and 10 days cultures were processed for IF and EM techniques.

Reagents

Cytochalasin B (molecular weight (MW) = 507·6), colchicine (MW = 459 13) and vinblastine sulphate (MW = 909·06) were obtained from Sigma. X537A, a calcium ionophore (MW = 590), was kindly supplied from Dr Ishii of Kyoto University and Dr Onodera of Tokyo University. The above drugs except colchicine were dissolved at appropriate concentrations in dimethylsulphoxide (DMSO). The final drug-containing medium was 0·1 to 0·5% (v/v) in DMSO (for details see Weber *et al.*, 1976; Weber, Bibring & Osborn, 1975; Poste & Nicolson, 1976, respectively). Colchicine was dissolved in physiological saline (Weber, Pollack & Bibring, 1975). The synthetic drug Mitsubishi No. 233 (Lot No. 801001), a calmodulin inhibitor (MW = 590·8), was generously donated by Dr Umezu of Mitsubishi Kasei Industries, Yokohama, Japan. To make this drug soluble, 1 N hydrogen chloride was added to an aqueous suspension of the drug until it became clear and then the solution was neutralized by 1 N sodium hydroxide without forming any precipitate.

Experimental treatments of cultured cells

(1) In order to determine whether fluorescent staining with our antibodies displayed arrays of MT, some of the cultured cells were added to (1) cytochalasin B (20 to 40 μM), (2) colchicine (10 to 20 μM) or (3) vinblastine sulphate (10 to 50 μM) and incubated at 37 °C in a CO_2 incubator for 1, 3 and 16 h, respectively. Control cultures were incubated in the medium containing 0·5% DMSO only. While cytochalasin B specifically disrupts microfilaments (actin) but not MT, the latter two agents are specific dissociators of MT.

(2) A group of cultures in MEM were given additional calcium chloride (0 to 1000 μM), X537A (0 to 100 μM) and/or Mitsubishi No. 233 (0 to 100 μM) at various concentrations as shown in Tables 1 and 2, and incubated in a CO_2 incubator for 6 h. At the end of the incubation cells were examined under a phase-contrast microscope and reactions were stopped by 2 mM EGTA (Rice & Green, 1979). Each experiment was performed in duplicate and repeated three times.

Skin specimens

Biopsy specimens taken from human and guinea-pig skin were immediately frozen in an acetone–dry ice mixture and stored at −80 °C until use. For IF staining 3 μ-thick frozen sections were prepared on a cryostat at −20 °C.

Fixation

An aliquot of trypsinized keratinocytes was fixed with 3·7% paraformaldehyde solution at room temperature for 5 min. Then, cells were washed with 10 mM phosphate-buffered saline pH 7·4 (PBS) twice by centrifugation, and smears were made using a cytocentrifuge apparatus (Tomy Industries, Tokyo, Japan). The smears were postfixed by chilled acetone for 1 min.

Cultured keratinocytes on coverslips were fixed with 3·7% paraformaldehyde solution at room temperature for 15 min followed by postfixation with chilled acetone for 5 min.

Cryostat sections were air-dried and used without fixation.

Immunofluorescent stain technique

All specimens were reacted first with monospecific anti-tubulin antibodies (1:2 to 5 diluted with PBS) for 30 min at 22 °C, rinsed in cold PBS and then stained with fluorescein isothiocyanate-conjugated swine anti-rabbit IgG antisera (1:20 diluted with PBS, total protein concentration 3·1 mg/ml, F/P molar ratio 1·65, Dakopatts Laboratories, Denmark) for 30 min at 22 °C. They were again rinsed in PBS and mounted in buffered 90% glycerol. Mounted slides were examined in a Nikon fluorescence microscope equipped with a 200 W high pressure mercury lamp and epi-illumination ultraviolet optics.

To examine specificity of immunoreactions specimens were stained with (1) pre-immune rabbit sera (1:20 diluted) and the conjugate and (2) anti-tubulin antibodies pre-absorbed by purified tubulin. Prior to staining the same amounts of antibodies and tubulin were mixed and kept at room temperature for 30 min.

Electron microscopy

Keratinocytes growing on plastic dishes were gently washed with warm PBS. They were fixed by 2·5% glutaraldehyde in 0·1 M phosphate buffer pH 7·4 at 4 °C for 2 h, washed in the buffer containing 7% sucrose and postfixed by 1% osmium tetroxide at 4 °C for 1½ h. After fixation cells were dehydrated in graded alcohol and embedded in Spurr. Ultrathin sections were cut in a Porter-Blum ultramicrotome, stained with uranyl acetate and lead citrate and examined in a Hitachi H-300 electron microscope.

RESULTS

Trypsinized cells

Smears of trypsinized keratinocytes stained with anti-tubulin antibodies apparently demonstrated a ring-shaped fluorescence (Fig. 1a) but by careful observation using differential focusing, several fluorescent spots representing the presence of free tubulin were observed beneath the cell membrane (Fig. 1b). The ring shape appeared to be due to the tangent plane effect.

Cultured cells

Isolated keratinocytes of 7- to 10-day cultures formed pseudo-colonies and some of them piled up. The lowermost cells were fully distended on the surfaces of coverslips: by IF microscopy examination fluorescent fibres extended well over the cytoplasm, spreading from the perinuclear area toward the cell margin (Fig. 2a). Fluorescence was concentrated around the nucleus. By differential focusing, cells piling up on the spread cells showed a homogeneous and, in part, a granular cytoplasmic fluorescence but not a fibrous pattern (Fig. 2b). Staining with pre-immune rabbit sera or monospecific antibodies pre-absorbed by tubulin gave negligible fluorescence in cells of both upper and lower layers (Fig. 2c).

Electron microscopically, cells of the lower two or three layers from the surface contained

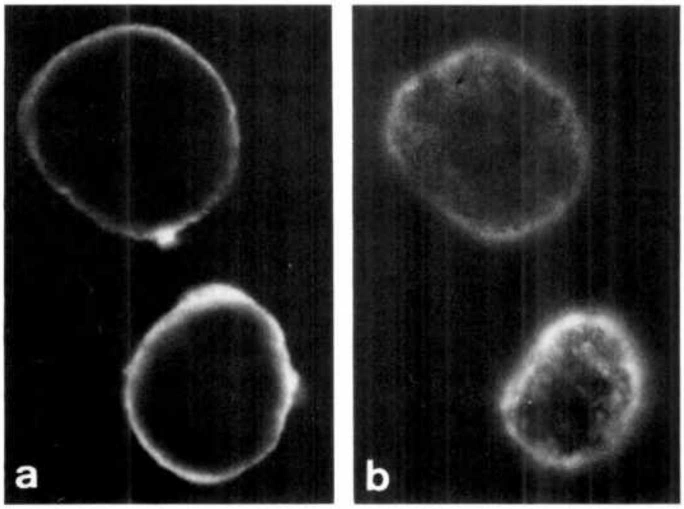

FIGURE 1. Immunofluorescence micrographs of smears of trypsinized guinea-pig keratinocytes showing (a) a ring-shaped fluorescence (× 2,850) and (b) by differential focusing, a reticular fluorescent pattern beneath the plasma membrane (× 2,850).

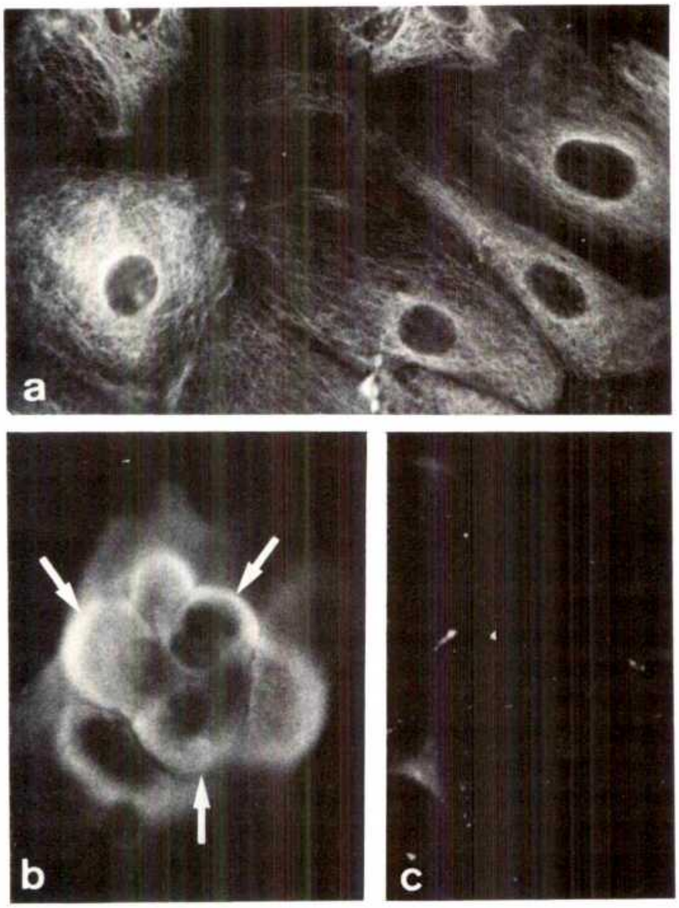

FIGURE 2. Immunofluorescence micrographs of guinea-pig keratinocytes in 7-day culture. (a) Adherent cells display organization of cytoplasmic microtubules. No fluorescence is seen in nuclei (× 685). (b) Focussing on cells in upper layers of a multilayered colony reveals a homogeneous and/or granular cytoplasmic fluorescence. Arrows indicate the margin of the cells (× 1200). (c) Specific fluorescence is almost completely abolished by pre-absorption of anti-tubulin antibodies with purified tubulin (× 620).

MT: they were well developed in adherent cells (Fig. 3a), while they appeared to become fewer and shorter in organization in upper layers (Fig. 3b), although measurements were not quantitative. More differentiated cells hardly displayed such structures.

Effects of experimental treatments on microtubular organization

(1) Treatment with cytochalasin B did not affect fibrous expression of cytoplasmic fluorescence (Fig. 4a). After treatment with colchicine and vinblastine sulphate, some of the adherent keratinocytes were somewhat reduced in size and gave a rounded cell shape. After staining with anti-tubulin antibodies the fluorescent fibres were completely abolished at the periphery of the cells, although perinuclear fluorescence remained visible showing a granular appearance (Fig. 4b). Paracrystals were formed in vinblastine-treated cells as has been shown by others (Weber, Bibring & Osborn, 1975) (Fig. 4c). DMSO alone did not change the network of fibrous fluorescence.

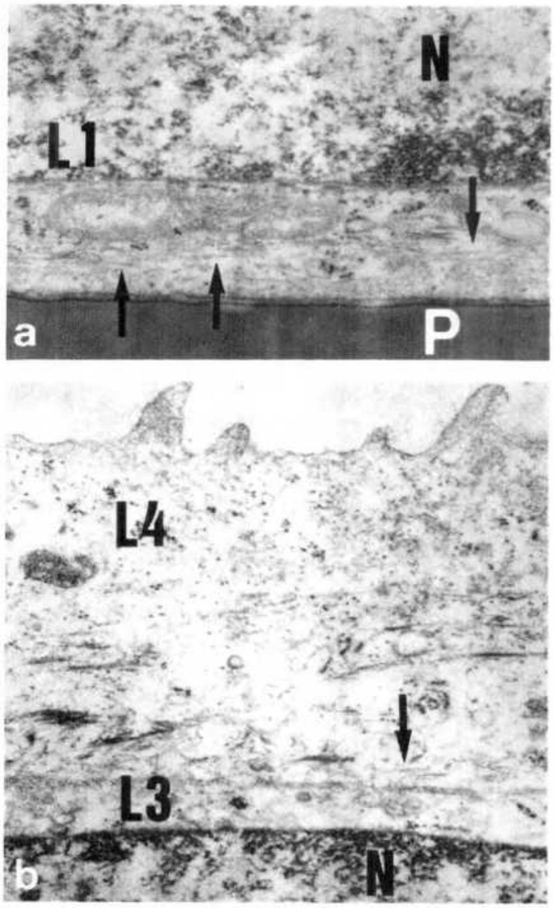

FIGURE 3. Electron micrographs of guinea-pig keratinocytes in 7-day culture showing (a) a lowermost cell (Ll) adhering to the plastic surface (P), containing bundles of microtubules (arrowed) in the cytoplasm (approximate diameter = 14 nm) (× 40,000) and (b) upper cells (L3, L4: the third and fourth layers, respectively) demonstrating few microtubules (arrowed) (× 32,000). L2 (the second layer) cells are not shown in this set of photographs. N indicates nucleus.

(2) The effect on cell shape and MT distribution was dependent upon both extracellular calcium ion (Ca^{2+}) and X537A levels (Table 1). MT were affected neither by Ca^{2+} alone nor by X537A alone, but were affected by the highest concentration of the latter (100 μM). Within the amounts used X537A was non-cytotoxic. Exposure to increased Ca^{2+} levels plus X537A caused apparent cell deformity and various degrees of MT disassembly. Many of the treated cells were isolated and showed elongated, irregular configurations: by IF microscopy these cells revealed only remnants of pieces of MT (Fig. 5a). To determine whether or not such Ca^{2+} effects on MT are mediated by calmodulin, a calcium-dependent modulator protein, we next used a calmodulin inhibitor (Table 2). The concomitant presence of Mitsubishi No. 233 during the incubation period obviously counteracted depolymerization effects of increased Ca^{2+} levels plus X537A (Fig. 5b).

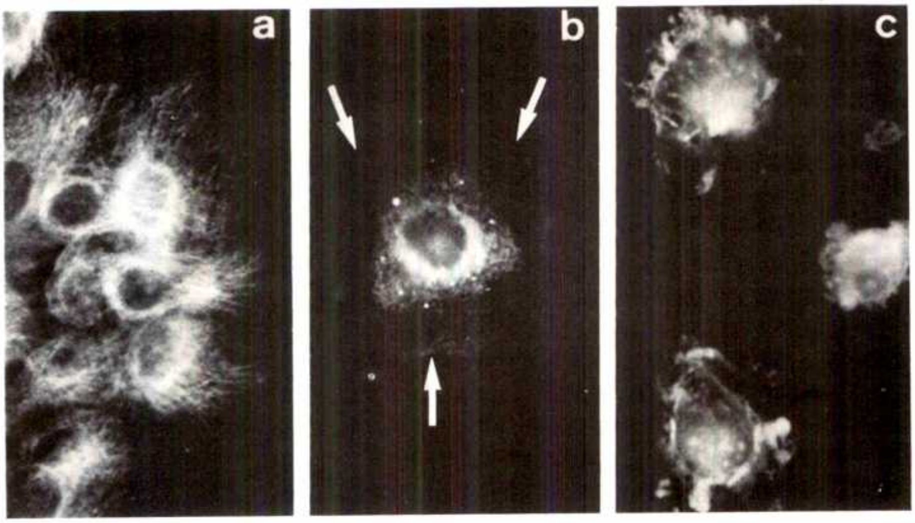

FIGURE 4. Effects of experimental treatment 1 on microtubular organization in cultured cells. (a) Cells exposed to cytochalasin B (40 μM) for 1 h show the normal fluorescent pattern (× 760). (b) Cells exposed to colchicine (10 μM) for 3 h demonstrate remnants of IF around the nucleus. Note disappearance of fibrous fluorescence at the periphery of the cell (arrowed) (× 570). (c) Cells exposed to vinblastine sulphate (10 μM) for 16 h are devoid of cytoplasmic fibres and some of them contain several fluorescent paracrystals in the cytoplasm (× 590).

TABLE 1. Effects of calcium ion (Ca^{2+}) and X537A on cell shape*/microtubular organization**

Ca^{2+} added (μM)	X537A (μM)			
	0	10	50	100
0	N/+	N/+	D_1/+	D_2/−
100	N/+	D_1/+	D_2/+	D_2/−
500	N/+	D_1/−	D_2/−	D_2/−
1000	N/+	D_2/−	D_2/−	D_2/−

* The cell shape was classified relatively into three degrees: N, apparently normal appearance; D_1, slight to moderate deformity; D_2, marked deformity.

** + indicates organization of microtubules (MT); − indicates disassembly of MT.

Cryostat sections

In both human and guinea-pig epidermis a homogeneous fluorescence was seen in the cytoplasm of basal to spinous layers and there was a granular fluorescence in the upper spinous to granular layers (Fig. 6a). No fibrous pattern was detected. The horny layer gave no fluorescence. Absorption of mono-specific antibodies by the tubulin preparation abolished fluorescence in all layers (Fig. 6b).

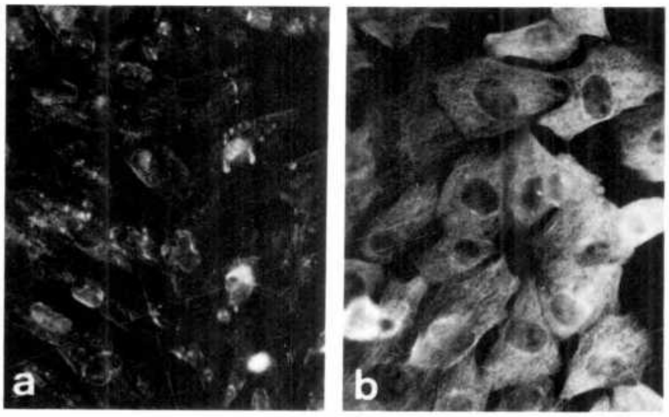

FIGURE 5. Effects of experimental treatment 2 on microtubular organization in cultured cells. (a) Cells preincubated for 6 h in the medium containing additional calcium chloride (1000 μM) and X537A (50 μM) demonstrate dramatic breakdown of the microtubular network (× 680). (b) Cells exposed to such treatment but in the presence of a calmodulin inhibitor (Mitsubishi No. 233) (20 μM) show apparently no alteration in the microtubular organization (× 680).

TABLE 2. Effects of calmodulin inhibitor (Mitsubishi No. 233) on cell shape and microtubular organization (MT) (for details see Table 1)

Ca^{2+} added (μM)	X537A (μM)	Mitsubishi No. 233 (μM)	Cell shape/MT
0	0	0	N/+
		20	N/+
		100	N/+
100	50	0	D_2/−
		20	N/+
		100	N/+
1000	50	0	D_2/−
		20	N/+
		100	N/+

DISCUSSION

A network of fibrous fluorescence was observed in cultured keratinocytes attaching to the substratum, while cells piling up on these cells displayed a homogeneous cytoplasmic fluorescence, suggesting that the basic arrangement of MT may be different in different layers of cultured cells. The fibrous fluorescence shown in adherent cells was disrupted by colchicine and vinblastine sulphate but was resistant to cytochalasin B, indicating that MT are organized in these cells (Osborn *et al.*, 1978; Osborn, Franke & Weber, 1977). The weak, granular remnants of IF in the cytoplasm after colchicine treatment may represent redistribution of microtubular proteins (Weber *et al.*, 1975). The fibres stained with our antibodies were distinct from

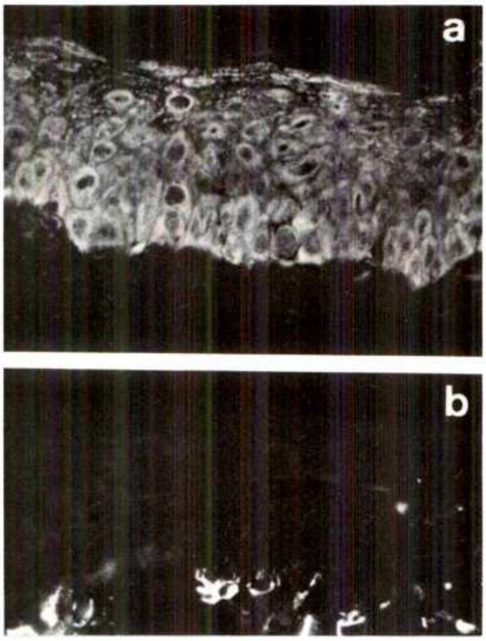

FIGURE 6. Immunofluorescence micrographs of cryostat sections of normal guinea-pig back skin showing (a) a homogeneous and/or granular cytoplasmic fluorescence from basal to granular layers (× 600) and (b) no detectable fluorescence when sections are stained with anti-tubulin antibodies preabsorbed with tubulin (× 600).

microfilaments, which are sensitive to cytochalasin B but not to colchicine, and also from tonofilaments, which are affected by neither of them. Such a distribution of MT in adherent cells seems to be primarily common to other cell lines, although it is debatable whether assembly of MT causes attachment of cells to the substratum or attachment causes MT assembly (Osborn & Weber, 1976).

We were not able to document the MT array in cells in upper layers or trypsinized cells. This is in part due to the round form of the cells in which overlapping images of MT would be present in many different focal planes (Osborn & Weber, 1976). However, EM studies which showed fewer MT or none in the upper cells would be in favour of the IF there representing reactants to depolymerized tubulin rather than to organized MT. Specificity of IF in those cells was confirmed by the absorption test. Other authors have shown that the MT network is disintegrated by exposure of cultured cells to trypsin (60 to 120 μg/ml) for a few minutes (Fuller & Brinkley, 1976), agreeing with our findings.

The mechanism for depolymerization of MT is unknown: it might result secondarily from detachment of cells from the substratum. However, we favour the possibility that MT disassembly is initiated by an influx of Ca^{2+}, since Ca^{2+} is known to control the MT assembly–disassembly process both *in vitro* (Weisenberg, 1972) and *in vivo* (Osborn & Weber, 1977; Schliwa *et al.*, 1981). Using detergent-extracted cells the latter investigators demonstrated that MT are highly labile to Ca^{2+}. In this study we have exposed intact cells to increased extracellular Ca^{2+} levels in the presence of a divalent ionophore (X537A), resulting in extensive fragmentation of MT networks. The degree of MT disassembly was dependent upon both Ca^{2+} and X537A concentrations, suggesting that ionophore-assisted Ca^{2+} entry into the cytoplasm indirectly affected MT. We could not neglect the possibility of release of Ca^{2+} from organelles such as mitochondria. These findings are consistent with those of Fuller & Brinkley (1976). Furthermore, such Ca^{2+} effects were completely blocked by a calmodulin inhibitor, suggesting that MT disassembly may be mediated by formation of Ca^{2+}–calmodulin complexes (Marcum *et al.*, 1978; Schliwa *et al.*, 1981). On the other hand, Ca^{2+} plays a key role in growth and differentiation of cultured keratinocytes (Hennings *et al.*, 1980). Rice & Green (1979) have suggested that at the ultimate stage of keratinization an increase in the intracellular Ca^{2+} level from either internal or external sources activates transglutaminase to begin the crosslinking process. Thus, it is assumed that MT disassembly may occur in association with differentiation of cultured keratinocytes.

Depolymerization of MT appears to take place also in the *in vivo* keratinization process. An electron microscopic study of biopsied normal skin specimens by Rupec (1974) has shown a short and tortuous organization of MT in basal cells but to a lesser degree in the spinous to granular layers. In this study, however, cryostat sections failed to show a fibrous fluorescence, although tubulin was demonstrated in all layers but the horny layer. This is perhaps because a process of freezing and thawing of the tissues may destroy the architecture of MT.

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Application of the tyrosinase assay to normal melanocytes in culture*

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SUMMARY

Using a special selection technique, normal guinea-pig melanocytes were maintained in highly purified but sparse cultures ($\sim 10^4$ cells/25 cm^2 culture vessel) which showed little proliferative activity. The applicability of the Pomerantz tyrosinase assay was tested in this *in vitro* model system using three different approaches, namely crude cell extracts and viable cell cultures either *in situ* or in suspension. The latter modifications both proved too insensitive, whereas crude cell extracts allowed accurate measurements of the basal tyrosinase activity and its stimulation by various agents. In unstimulated cultures basal tyrosinase activities ranged from 30% to 700% (mean 260%) above the blank values; intra-assay and inter-assay variability were 4·2% and 77·5%, respectively. Stimulation with α-MSH (10^{-5} M, 10^{-6} M), β-MSH (10^{-5} M), choleratoxin (10^{-11} M) and cAMP (10^{-4} M) plus theophylline (10^{-4} M) resulted in an increase of tyrosinase activity 30–65% above basal values. Melanotropin potentiating factor (10^{-8} M) enhanced the effects of α-MSH (10^{-6} M) by 20%. This assay modification provides a sensitive tool for comparative studies of melanogenesis in normal melanocytes, malignant melanocytes and otherwise altered melanocytes.

A radio-enzymatic method for the measurement of tyrosinase, the key enzyme of melanogenesis (Prota, 1980), was introduced by Pomerantz (1964) and has been extensively employed in studies of melanin synthesis, using tissue homogenates or cultured melanoma cells as substrates. Normal mammalian melanocytes, the proper object for investigation of the biology of melanin synthesis, have hitherto not been available for this assay due to the lack of an effective isolation and culture technique to allow melanocytes to be grown in quantities sufficient for biochemical analysis (Fritsch *et al.*, 1981).

We have established a simple selection technique to prepare primary guinea-pig melanocyte cultures of high purity (Fritsch, Tappeiner & Huspek, 1979). Extensive experiments designed to stimulate these cultures to satisfactory growth failed. This precluded the application of biochemical methods with low sensitivity. A number of modifications of the Pomerantz assay

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were tried to determine its lower limits of sensitivity, i.e. the lowest number of cells permitting accurate measurements of their tyrosinase activity (TA). In this study we show that a minimum of 10^4 melanocytes is sufficient for the measurement of unstimulated basal and stimulated TA. Our model system should thus provide a basis for comparative studies of melanogenesis in normal and chemically altered melanocytes (Eisinger & Marko, 1982) and also in melanoma cells.

METHODS

Design of experiments

Primary melanocyte cultures were subjected to the tyrosinase assay by three different approaches: as crude cell extracts, and as viable cells either *in situ* or in short-term suspension cultures. To test the assay system, the effects of a number of agents known to stimulate TA in different *in vivo* or *in vitro* systems were studied.

Melanocyte cultures

Cultures were prepared from the epidermis of the ears of black wild type guinea-pigs using a selection technique based on the omission of magnesium in the attachment phase (Fritsch *et al.*, 1979). Epidermal cell suspensions obtained by trypsinization (0·25% trypsin in phosphate-buffered saline, PBS) at 37°C for 30 min, subsequent mechanical separation of dermis and epidermis and gentle teasing of the epidermis in PBS, were plated in magnesium-free Eagle's MEM supplemented with 20% dialysed fetal calf serum in 25-ml Falcon flasks. After overnight incubation at 37°C, the cultures were rinsed thoroughly with PBS to remove unattached viable keratinocytes and maintained in normal Eagle's MEM containing 10% fetal calf serum. By this method, the average melanocyte yield was about 10^4 from an inoculum of 7 to 8×10^6 epidermal cells per 25-ml Falcon flask. The cultures consistently contained more than 90% melanocytes. All tissue culture reagents were purchased from Flow Laboratories, Irvine, U.K.

Cell counting

The total number of melanocytes per flask was determined from *in situ* cell counts in twenty-five randomly selected microscopic fields (0·49 mm^2), which were extrapolated to the total growth area of the flask (25 cm^2).

Tyrosinase assay

Principle. The Pomerantz assay (Pomerantz, 1964) uses tritiated L-tyrosine [^3H]T for quantifying the initial step catalysed by tyrosinase, (monophenol, dihydroxyphenylalanine: oxygen oxidoreductase, EC 1.14.18.1.), the hydroxylation of L-tyrosine to L-dopa. The rate of ^3H thereby released as tritiated water (^3HOH) is proportional to the rate of hydroxylation and thus represents a measure of TA.

General. All experiments were carried out on 4- to 7-day-old primary melanocyte cultures. All measurements were performed in duplicate. Immediately prior to use [^3H]T (specific activity 50 Ci/mmol, The Radiochemical Centre, Amersham, U.K.) was dried under a nitrogen stream to remove residual ^3HOH.

Tyrosinase assay in crude cell extracts. Melanocyte cultures were trypsinized (0·25%, 20 min, 37°C), washed (PBS) and lysed (1% Triton X-100, 1 h, 4°C) followed by sonication (10 sec).

The resulting crude extract was incubated (37°C) with [^{3}H]T (10 μCi = 0·2 nmol) and a catalytic amount of L-dopa (0·1 nmol, Sigma, Munich, FRG) required for the oxidation of L-tyrosine. The reaction mixture was made up with phosphate buffer (0·08 M, pH 6·8) to a total volume of 280 μl. The incubation was stopped with metaphosphoric acid (final concentration 2·0%) after 2 h. Unreacted [^{3}H]T and its reaction products were removed by charcoal adsorption: 500 μl of a Norit A (Sigma, Munich, FRG) charcoal slurry (10% w/v in 0·1 M citric acid) was mixed into each incubation tube for 15 min and then spun down (1500 ***g***, 10 min). Aliquots of the supernatant were counted for their ^{3}HOH contents in a liquid scintillation counter (1215 Rackbeta, LKB-Wallac, Turku, Finland). In order to facilitate comparison of different experiments and modifications, basal TA values were expressed as per cent above blank values (TA% = enzymatic ^{3}HOH production per 10^4 melanocytes/non-enzymatic ^{3}HOH production × 100).

Tyrosinase assay in viable cells. Melanocyte cultures were exposed to FCS-supplemented medium containing [^{3}H]T (1 μCi/ml) for 20 h. The medium was then removed, subjected to charcoal-adsorption as described above, and its ^{3}HOH content measured. As a modification aimed at increasing the ratio of cell number to volume of medium and at minimizing cell counting errors, melanocytes from several cultures were pooled, distributed in appropriate aliquots and kept suspended in roller bottles during incubation with [^{3}H]T (1 μCi/ml). The subsequent procedures were the same as above.

Blanks were run with each assay to correct for non-enzymatic formation of ^{3}HOH: incubation mixtures without crude cell extracts, or [^{3}H]T containing media incubated in the absence of melanocytes. In another set of experiments, non-specific enzymatic production of ^{3}HOH was ruled out in fibroblast cultures.

Stimulation of TA

Cultures were incubated with FCS-supplemented medium containing one of the following agents for 20 h prior to the measurement of TA: α-MSH (10^{-6} M, 10^{-5}M), α-MSH (10^{-6} M) plus melanotropin potentiating factor (MPF) (10^{-8} M), β-MSH (10^{-5} M), choleratoxin (10^{-11} M), or cAMP (10^{-4} M) plus theophyllin (10^{-4} M). In the modification on viable cells the culture medium also contained [^{3}H]T. All reagents were obtained from Sigma (Munich, F.R.G.) except theophylline (Serva, Heidelberg, F.R.G.), β-MSH and MPF (Bachem, Bubendorf, Switzerland).

Statistical analysis

The response of TA to stimulation was assessed by the sign test using the single values of the replicate determinations for computation. Comparisons were made between basal TA and TA after stimulation with α-MSH, β-MSH, choleratoxin and cAMP plus theophyllin. Furthermore, the effect of MSH alone was compared with the combined effect of MSH plus MPF. The intra-assay variability (i.e. within-batch precision) was calculated from the mean standard deviation (s.d.) of forty duplicate determinations and expressed as the coefficient of variation (%). The inter-assay variability (i.e. between-batch precision) was calculated from the mean s.d. of the basal values from sixteen assays and expressed as the coefficient of variation (%).

RESULTS

Basal TA

The assay modification using viable cells *in situ* consistently gave basal values indistinguishable from the blank values, whereas with viable cells in suspension basal TA was measurable in all but one of the experiments (Table 1). The modification in crude cell extracts proved to be the superior approach, resulting invariably in basal TA values which ranged significantly above their corresponding blank values.

With crude extracts the correlation between number of melanocytes assayed and TA recovered was strictly linear ($r=0{\cdot}99$, $2P<0{\cdot}001$; Fig. 1). The ratio of enzymatic to non-enzymatic ^{3}HOH release, obviously the delimiting factor of the assay sensitivity, is shown to be approximately 4:1 at a total number of 10^4 melanocytes, thus clearly granting satisfactory determination of TA from this modicum of cells. The intra-assay variability was consistently found to be very low (mean 4·2%), which contrasted with a high inter-assay variability (77·5%).

Stimulation of TA

Each of the substances tested induced a significant stimulation of TA, as measured in the crude extracts (Table 2). The increases fell fairly uniformly into a range of 30–60% above basal TA for all agents, the highest values being observed for cAMP + theophylline. Analogous to the high inter-assay variability of basal TA among the individual primary melanocyte cultures, the response of the cells to TA stimulatory agents varied considerably from culture to culture. It is

TABLE 1 Basal tyrosinase activity per 10^4 M, expressed as percentage increase above values of non-enzymatic ^{3}HOH production

		Viable cells	
	Crude extract	*in situ*	in suspension
Mean ± s.e.m	258 ± 50·1 %	5 ± 5·7 %	67 ± 15·3 %
Range	29–682 %	−7–31 %	−1–214 %
Number of assays performed	16	6	5

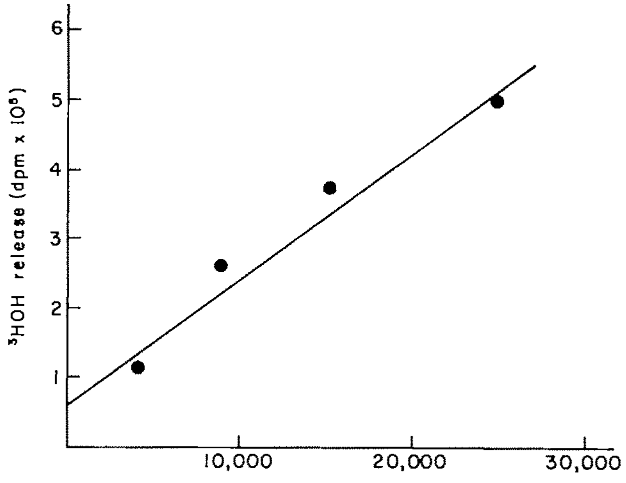

FIGURE 1. Tyrosinase activity as a function of number of melanocytes assayed. Coefficient of correlation ($r=0{\cdot}99$, $2P<0{\cdot}001$). The intersection of the regression line with the ordinate represents the amount of non-enzymatic ^{3}HOH production.

TABLE 2. Stimulation of tyrosinase activity

Agents tested	Mean ± s.e.m.	Range	N		$2P$
α-MSH (10^{-6} M)	28·7 ± 9·35*	6·0–94·4	9	<	0·01
α-MSH (10^{-5} M)	25·3 ± 21·42*	2·2–161·1	7	<	0·01
β-MSH (10^{-5} M)	31·9 ± 7·61*	14·5–48·9	4	<	0·05
Choleratoxin (10^{-11} M)	41·9 ± 13·74*	17·7–118·2	7	<	0·01
cAMP (10^{-4} M) + theophylline (10^{-4} M)	65·3 ± 30·63*	17·0–153·6	4	<	0·05
α-MSH (10^{-6} M) + MPF (10^{-8} M)	19·9 ± 3·93**	11·0–29·9	4	<	0·05

* Percentage increase above basal TA.
** Percentage increase above MSH-stimulated TA.
N = number of experiments performed in duplicate.

noteworthy that raising the concentrations of α-MSH from 10^{-6} to 10^{-5} M was not followed by any further significant increase of TA. Melanotropin potentiating factor (10^{-8} M) enhanced the effects of α-MSH (10^{-6} M) significantly by 19·9% (Table 2), whereas it failed to stimulate TA on its own (data not shown).

The assay modification using viable cells *in situ* failed to monitor any sizeable stimulatory effect by any of the substances tested (data not shown). Viable cells in suspension exposed to cAMP in one experiment responded with an increase of TA of 150%. In striking contrast, stimulation with α-MSH (10^{-6} M) in three experiments failed to produce any increase (3 ± 3·4%, mean ± s.e.m.).

DISCUSSION

The above data demonstrate that the Pomerantz tyrosinase assay can be applied to numbers of cultured melanocytes as small as 10^4, a cell number which can easily and reproducibly be achieved with the melanocyte selection technique used (Fritsch *et al.*, 1979). The assay is sensitive enough to measure the basal TA and its increase by the stimulatory effect of various agents.

Of the three modifications tested, the TA measurement in crude cell extracts unequivocally proved the most sensitive and was thus the method of choice. Both modifications based on the use of viable cells gave consistently higher and more variable blank values resulting in a low degree of sensitivity. The causes of the higher blank values in these modifications are not clear, but are likely to depend on imponderable factors in the culture conditions which cannot be matched in the set-up of the blanks, such as fluctuations in pH of the medium. Compared with viable cells *in situ* which were completely unsuitable for TA determination, the modification with viable cells in suspension was still appropriate for detecting basal TA levels. However, in this system MSH failed to induce TA stimulation, whereas cAMP, the intracellular mediator for the MSH response, led to a marked activation of TA. These apparently discrepant results might be attributed to the absence or incomplete restoration of MSH surface receptors following the trypsinization process (Varga *et al.*, 1974).

The accuracy of the modification based on crude cell extracts is expressed by the linear correlation between cell number assayed and TA measured, and by the low intra-assay variability, demonstrating the high reproducibility of replicate determinations in individual crude extracts. In sharp contrast, the high inter-assay variability reflects striking differences between individual melanocyte cultures, a finding which is likely to be related to age, colour and genetic dispositions of the animals used and to imponderable culture factors.

The Pomerantz assay has hitherto been applied to skin homogenates (Pomerantz, 1969; Pomerantz & Ances, 1975), to hair follicle scrapings (Logan & Weatherhead, 1978; Weatherhead & Logan, 1981) and particularly to melanoma cells (Pawelek, 1976; Lande *et al.*, 1981). Unlike cultured normal melanocytes all of these systems provide abundant pigment cells for biochemical analysis, but are still unsatisfactory tools for pigment cell research for several reasons. Skin homogenates do not allow more than rough estimations of pigmentary events occurring *in vivo*; hair follicle scrapings are limited to studies in mammalian species showing colour changes of the pelage. Therefore, most of our knowledge of melanin synthesis has been derived from animal melanoma cells (Pawelek, 1976).

Our culture model system offers for the first time the possibility of re-assessing some of these findings in normal unaltered melanocytes. In this paper, we have shown that the major steps of control of melanin synthesis, as demonstrated in melanoma cells, can be confirmed with stimulation of TA following exposure to α-MSH (Pawelek *et al.*, 1973; Wong *et al.*, 1974; Lee & Lee, 1977; Fuller & Viskochil, 1979), β-MSH (Lande *et al.*, 1981), cAMP (Johnson & Pastan, 1972; Wong & Pawelek 1973; Lee & Lee, 1976) and choleratoxin (O'Keefe & Cuatrecasas, 1974). In addition, our model system proved capable of demonstrating the actions of MPF, the C-terminal tetrapeptide of β-lipotrophin (Carter, Shuster & Morley, 1979), which has been recently reported to potentiate the effects of MSH on reptilian melanophores (Carter & Shuster, 1979) and on hair follicle melanocytes of the Siberian hamster (Logan *et al.*, 1981), but not on B16 mouse melanoma cells (Johnson *et al.*, 1980). Our results add further evidence to the speculation that regulation of MSH-mediated melanogenesis by MPF may be a general and important feature in mammals.

We have shown that even sparse cultures of normal melanocytes are amenable to restricted biochemical analysis, as is the measurement of TA. Nevertheless, for more extensive biochemical investigations on melanocytes improvement of the cell yield is felt to be a prerequisite. A promising approach to this problem has recently been reported by Eisinger & Marko (1982), who accomplished selective adhesion and promotion of proliferation of normal cultured human melanocytes by the concomitant use of the tumour promoter phorbol-myristate-acetate, and choleratoxin, a stimulator of adenylate cyclase. However, it remains to be seen whether these artificial culture conditions alter the metabolic activity of the cells as well as their ability to react to the physiological signals controlling melanin synthesis. Our highly reproducible and sensitive adaptation of the tyrosinase assay to crude cell extracts offers a tool to enable the primary events of melanogenesis in these chemically altered melanocytes to be compared with those in definitely normal melanocytes.

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Photobiological activity of suction blister fluid from patients treated with 8-methoxypsoralen

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SUMMARY

Suction blister fluid was collected from normal human volunteers before (SBF) and 2 h after (SBF 8-MOP) oral 8-methoxypsoralen (0·6 mg/kg) ingestion without irradiation. In SBF 8-MOP the concentration of 8-MOP was 150 ng/ml, and fluorescent metabolites were also present. The toxicity of SBF 8-MOP was then determined with and without UV-A irradiation in the diploid strain D_7 of the yeast *Saccharomyces cerevisiae* which is suitable for the detection of lethal, mutagenic and recombinogenic events. It was compared with that of SBF, SBF with 8-MOP added *in vitro* (150 ng/ml) and 8-MOP (150 ng/ml) in water. SBF showed no effect with UV-A doses up to 360 kJ m^{-2}; SBF 8-MOP showed a slight decrease in survival and a dose-dependent increase in nuclear mutations, mitotic gene conversion and crossing over; SBF with 8-MOP added *in vitro* showed increased photobiological activity compared with SBF 8-MOP. This photobiological activity was increased by a factor of six when using 8-MOP in water. These results indicate a different bioavailability of 8-MOP in water and in interstitial fluid containing proteins. The metabolites of 8-MOP in human skin did not increase the photobiological activity of the drug. We conclude that the 8-MOP present in human skin during PUVA therapy is mutagenic and recombinogenic for eukaryotic cells.

8-Methoxypsoralen (8-MOP) and its metabolites can be detected in the fluid of suction blisters from the skin of human volunteers, 2 h after 8-MOP oral intake (Blais *et al.*, 1982; Herfst, Edelbroek & De Wolff, 1980). The biological activity of 8-MOP and its metabolites in this medium has now been investigated using a well-known eukaryotic cellular system, the yeast *Saccharomyces cerevisiae*.

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METHODS

Suction blister fluid (SBF) (0·5–1 ml) was collected from twenty normal human volunteers by mild suction (175 mmHg) using the device described by Blais *et al.* (1982). This device raises suction blisters of 1·8 cm diameter on the inner face of the thigh. It has been shown that under these experimental conditions the biochemical composition of blister fluid is similar to that of interstitial fluid and contains mainly proteins and lipids (Vermeer, Reman & van Gent, 1979; Volden *et al.*, 1980). The suction apparatus was applied to the patient's skin before 8-MOP ingestion for the first blister and for the second one at the time of 8-MOP ingestion (0·6 mg/kg body weight). The dermo-epidermal separation started 2 h later. No UV-A irradiation was performed. Blister fluid was stored at −20°C until further analysis.

For each sample, the fluorescence emission was checked as previously described (Blais *et al.*, 1982). In eighteen cases, the fluorescence excitation and emission spectra were found to be similar to those described in our previous paper (Blais *et al.*, 1982). These eighteen samples were then pooled for analytical and biological experiments.

Analytical measurements

We used a Hewlett Packard mass spectrometer coupled with a gas chromatograph type HP 5992 B equipped with a gas capillary 25 m long with an internal diameter of 0·3 mm and coated with silicone polymer (SE 52). Helium filtered on a molecular sieve was used as carrier gas at a flow rate of 2 ml/min. Instrument temperatures were: injector 180°C; oven from 180 to 280°C (with temperature programming 6°C per min). The mass spectrometer was used under the following conditions: accelerating voltage 1800 V; ionization energy 70 eV.

A 200-μl aliquot of the pooled suction blister fluid from eighteen volunteers was analysed. These 200 μl of blister fluid, acidified with 20 μl of 0·5 N hydrochloric acid and spiked with 50 μl of a 1 μg/ml ethanolic solution of 5-MOP as internal standard, were extracted by 1 ml of ether. After centrifugation the organic phase was evaporated under a gentle stream of nitrogen, and the residue was diluted with 50 μl of ethyl acetate and one microlitre was injected into the chromatograph.

The level of 8-MOP in the biological material was calculated from a calibration curve obtained by adding known amounts of 8-MOP (1 to 50 ng/200 μl) and a constant amount of 5-MOP (50 ng/200 μl) to blister fluid taken before ingestion of 8-MOP.

Biological studies

The photobiological activity of SBF was assessed using the diploid strain of the yeast *Saccharomyces cerevesiae D_7* (Averbeck & Moustacchi, 1979). This strain permits the simultaneous study of the induction of reverse mutation, mitotic gene conversion and mitotic crossing over (Zimmermann, Kern & Rasenberger, 1975). The induction of cytoplasmic 'petite' mutation was also determined, using the tetrazolium overlay technique (Ogur, John & Nagai, 1957).

The culture medium conditions, the detection of genetic events and the irradiation procedures were as previously described (Averbeck & Moustacchi, 1979). All experiments were performed in triplicate. We used stationary phase cells obtained after 48-h incubation at 30°C in complete yeast extract glucose medium (Averbeck & Moustacchi, 1979). The cells were washed twice in distilled water (Biosedra) and resuspended in SBF taken 2 h after 8-MOP ingestion (SBF 8-MOP) at a final concentration of 2×10^7 cells/ml, and incubated for 30 min in the dark at room temperature. Different aliquots were exposed to several doses of 365 nm irradiation. One aliquot was taken as control.

Cells at the same concentration prepared in distilled water with or without an equal amount of 8-MOP (150 ng/ml) (H_2O + 8-MOP) were exposed to UV-A in the same conditions, as well as SBF taken before 8-MOP ingestion (SBF) and SBF taken before ingestion to which was added 8-MOP (150 ng/ml) (SBF + 8-MOP). In this latter experiment the incubation time of cells with SBF + 8-MOP was 30 min for one aliquot and 48 h for another.

Irradiation was performed with a Philips HPW 125 culot Edison, high pressure mercury lamp, emitting mainly at a wavelength of 365 nm and at a dose rate of 1·2 kJ m^{-2} min^{-1}, as measured by Black-ray J 221 long-wave ultraviolet intensity meter (Ultraviolet Products Inc., San Gabriel, CA 91778).

After irradiation, cell samples were diluted and plated on selective and non-selective growth media. For statistical analysis Student's t tests were used and $P < 0{\cdot}05$ was used as the level of significance.

RESULTS

SBF after oral ingestion of 8-MOP

Diploid cells in the presence of SBF after oral 8-MOP showed a slight but significant decrease in survival, i.e. colony forming ability (Fig. 1), and an increase in gene conversion (Fig. 2), reverse mutation (Fig. 3) and mitotic crossing over (Fig. 4) after exposure to UV-A doses up to 360 kJ m^{-2}. The frequency of the genetic effects was more than ten times higher than the spontaneous background. The fluorescent signal of metabolites was formed as described by Blais *et al.* (1982) and the concentration of unmetabolised 8-MOP was 150 ng/ml. The following controls were performed.

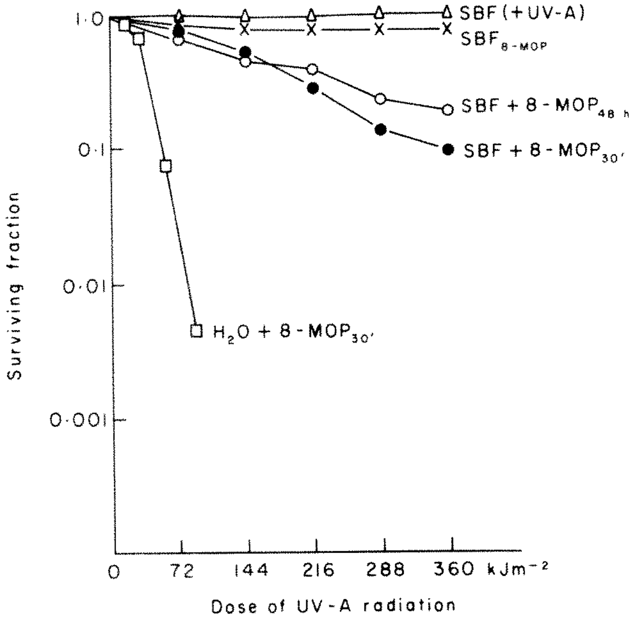

FIGURE 1. Effects on survival of yeast cells as a function of UV-A dosage.

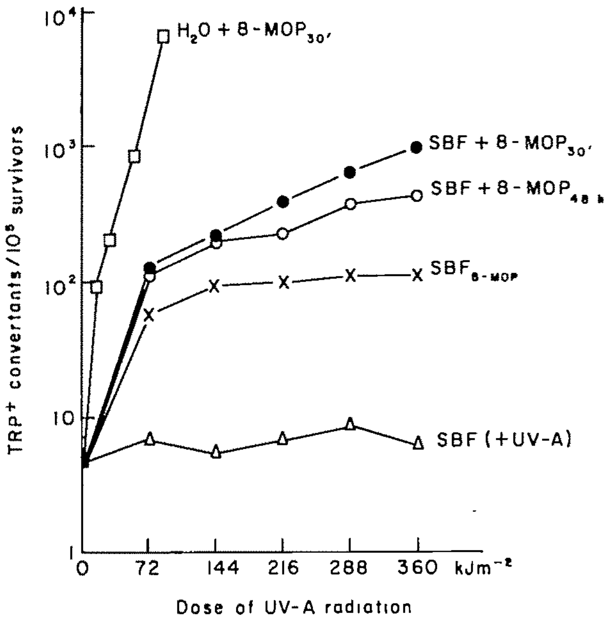

FIGURE 2. Effects on gene conversion as a function of UV-A dosage.

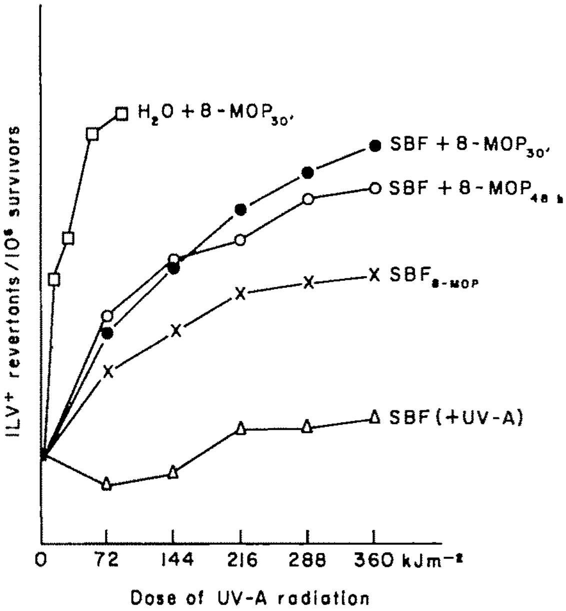

FIGURE 3. Effects on reverse mutation as a function of UV-A dosage.

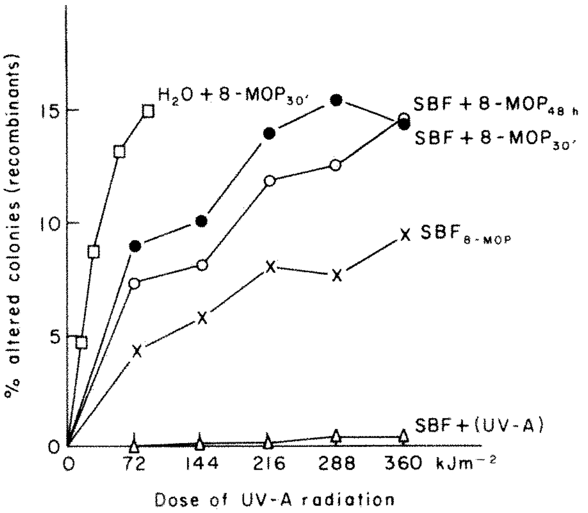

FIGURE 4. Effects on mitotic crossing-over as a function of UV-A dosage.

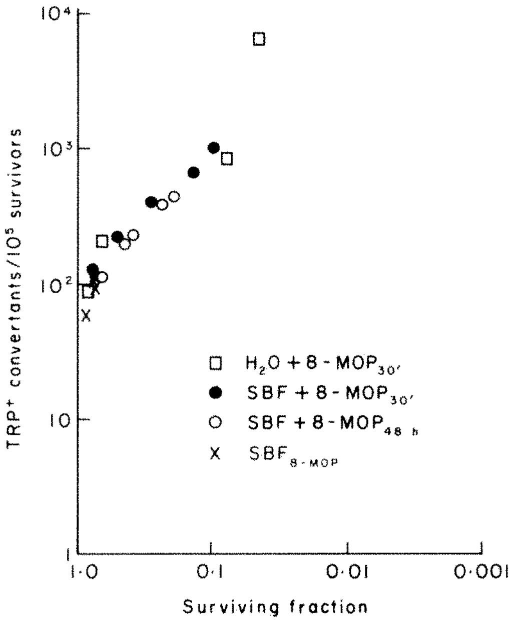

FIGURE 5. Effects on gene conversion as a function of survival.

SBF$_{8\text{-MOP}}$ = suction blister fluid taken 2 h after oral ingestion of 8-MOP.

SBF + 8-MOP$_{30'}$ = suction blister fluid taken before 8-MOP ingestion and pre-incubated for 30 min with 150 ng/ml (final concentration) 8-MOP before incubating yeast cells in its presence.

SBF + 8-MOP$_{48\ h}$ = suction blister fluid taken before 8-MOP ingestion and pre-incubated for 48 h with 150 ng/ml (final concentration) 8-MOP before incubating yeast cells in its presence.

SBF (+ UV-A) = cells in SBF taken before 8-MOP ingestion.

H_2O + 8-MOP$_{30'}$ = 8-MOP was added to a yeast cell suspension at 150 ng/ml final concentration in sterile water before incubation of cells.

SBF with later addition of 8-MOP (150 ng/ml)

Using this incubation medium we observed an important increase in the induction of lethal effect (Fig. 1) as well as in the considered genetic effects (Figs 2–4) by a factor of up to ten. However, when the incubation time of 8-MOP in SBF was increased from 30 min to 48 h before incubation of cells, the lethal (Fig. 1) and genetic effects (Figs 2–4) were slightly reduced, suggesting that the binding of 8-MOP to proteins of SBF increased with time and reduced the bioavailability of 8-MOP for the cells.

Distilled water with added 8-MOP (150 ng/ml)

In conditions used normally for *in vitro* studies (Averbeck & Moustacchi, 1979) (i.e. in the absence of human proteins) the lethal (Fig. 1) and genetic effects (Figs 2–4) were found to be much more striking (× 200) than in the presence of SBF. In other words only one-sixth of the dose of UV-A was needed for 10% survival and a revertant frequency of 10^{-2} in the case of 8-MOP (150 ng/ml) in water than in the case of 8-MOP (150 ng/ml) in SBF.

SBF before 8-MOP intake

Without 8-MOP, SBF had no effect on survival (Fig. 1), gene conversion (Fig. 2) and mitotic crossing over (Fig. 4) up to a UV-A dose of 360 kJ m^{-2}, and only a very slight but significant increase in reverse mutation (Fig. 3). No effect was observed in the absence of UV-A irradiation.

When plotting the frequency of reverse mutation and gene conversion induced as a function of survival, the efficiency of 8-MOP present in the different fluids appeared to be exactly the same (Fig. 5), underlining the reproducibility of the method.

The induction of cytoplasmic 'petite' mutation after exposure to 360 kJ m^{-2} in the presence of SBF after oral 8-MOP was barely significant. However, it was increased five-fold when using SBF with added 8-MOP (150 ng/ml) and also increased eight-fold with 8-MOP (150 ng/ml) in water.

DISCUSSION

Our results show that photobiological activity can be detected in suction blister fluid (representing interstitial fluid of human skin) 2 h after 8-MOP oral intake using a eukaryotic cell system as the biological probe. The method used constitutes an original way of studying the bioavailability of 8-MOP *in vivo* in human skin.

A comparison of the results obtained with the same quantity of 8-MOP in SBF and in water indicates that 8-MOP is apparently more effective in water than in SBF. This is probably related to the binding of 8-MOP to proteins (Veronese *et al.*, 1978; Veronese *et al.*, 1979; Bevilacqua *et al.*, 1981) leading to a decrease in bioavailability of 8-MOP. This decrease is illustrated by the fact that SBF after 8-MOP ingestion is much less photoactive than the same quantity of 8-MOP added *in vitro* to SBF taken before 8-MOP ingestion. This suggests that 8-MOP protein binding is stronger after *in vivo* passage than after *in vitro* incubation. Moreover, the binding of 8-MOP with proteins can be increased *in vitro* by increasing the time of contact between SBF and 8-MOP as suggested by the decrease of photobiological activity observed after 48 hours' incubation as compared with 30 min incubation.

The decrease in photobiological activity of SBF after oral 8-MOP compared with 8-MOP added *in vitro* to SBF shows that the 8-MOP metabolites present in the human skin 2 h after 8-MOP ingestion probably did not increase the photobiological activity in our experimental conditions. Recently, it has been suggested that this may be due to their contribution to light filtering (Blais *et al.*, 1982).

Our results confirm that 8-MOP inhibits colony forming ability and is mutagenic and recombinogenic in a diploid eukaryotic cell system such as *Saccharomyces cerevisiae*. This is true not only for several *in vitro* systems when using 8-MOP in free solution (Scott, Pathak & Mohn, 1976) but also when using human suction blister fluid taken 2 h after 8-MOP ingestion (i.e. in therapeutic conditions). From these results a mutagenic and recombinogenic activity of 8-MOP on human skin cells can be expected.

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The unit area trichogram in the assessment of androgen-dependent alopecia

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SUMMARY

The unit area trichogram was defined in the frontal and occipital areas of ten normal men and ten normal women and the results compared with the findings in ten male and fifteen female patients with androgenic alopecia. The epilation technique provided accurate data of normal hair density (higher than previously reported) and the phases of hair growth. The measurement of hair diameter showed that of 7603 hairs, 1241 were less than 40 μm in diameter, of which only 21 were more than 80 mm in length. Variation in the diameter of individual hair fibres was recorded. The number of hairs less than 40 μm diameter per cm^2 of scalp was increased in both men and women with androgenic alopecia. It was concluded that the number of hairs greater than 40 μm in diameter per cm^2 (meaningful density) reflected most closely the amount of hair usefully contributing to an individual's clinical appearance.

The development of drugs which will improve the status of scalp hair has been limited by difficulties in detecting and measuring the response to such preparations. The aim of therapy is to bring about recognizable changes in texture and appearance, but at present long-term treatment is necessary before either the changes can be detected, or it can be assumed they will never materialize. Even when a subjective improvement can be recognized, comparison between therapeutic regimens is difficult and necessitates the examination of a large number of patients to achieve statistical significance. A clinically objective alternative is to use measurements which relate to hair quality, and which if measured precisely, can indicate by early numerical changes whether or not improvement or deterioration is taking place. Therefore, therapeutic success or failure may be anticipated before gross visual and textural evidence appears.

A method for obtaining such precise values which can be used to evaluate the current hair

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status is described below. The application of the technique is demonstrated by comparison of normal male and female values with those obtained from males and females with androgenic alopecia.

METHODS

Ten normal male (18–32 years, mean 22) and ten normal female (17–29 years, mean 21) Caucasians, who had experienced no noticeable episodes of hair thinning, excessive hair shedding, recent illness or general health disturbances were selected as control groups. No menstrual cycle irregularities for at least 1 year prior to sampling had been reported by any of the females. Two had been taking oral contraceptives over the previous 2 years, while the remainder had never taken any such preparation. Clinical examination revealed no evidence of hair disorder in either male or female subjects.

Ten male patients (20–39 years, mean 24) and fifteen female Caucasian patients (17–38 years, mean 23), who had recognized gradual thinning of scalp hair for 4–15 years (mean 7, males) and 2–12 years (mean 6, females), in either a diffuse or localized pattern, were compared with the controls. All the male patients presented with a clinical diagnosis of androgenic alopecia (male pattern baldness) of various degrees of involvment. The female patients all presented with diffuse thinning characteristically associated with female androgenic alopecia, while not exhibiting any other clinical evidence of endocrine abnormalities. Thyroid and haematology profiles were all normal and no subject had suffered a general health disturbance or illness necessitating medical treatment within the previous year. Six female patients were currently taking the oral contraceptive (two combined, four progestogen only), while two had an IUD. Subjects with alopecia areata, post-partum or post-febrile hair thinning, transient alopecias and cicatrical alopecias were excluded from the study.

All subjects followed the same standardization procedure over the 2 days prior to sampling. This involved washing the hair in the morning of the first day (day 1), followed by combing the hair four times (morning, midday, afternoon and evening). The combing procedure was repeated the following day (day 2). On day 3, the hair was combed in the morning, and 2 hours later hair samples were taken from two sites, one in the frontal area, 10 to 30 mm right of the mid-line and 35 to 55 mm from the frontal periphery, and the second in the occipital area, 0 to 30 mm right of the mid-line and 80 to 110 mm from the occipital periphery. The mid-line was defined as that extending from the nose to the occipital protuberance.

Each area to be sampled was marked with a felt tip pen, through a template containing a precision drilled hole. The final area sampled varied from 35 mm^2 to 44 mm^2 due to the type of fibre tip pen employed. However, the exact dimensions of the area were determined by macrophotography, using a Canon F1n camera, fitted with a Canon 100 mm macro lens set at 1:1. From the negative, a black and white photograph was reproduced at five times magnification, and the area was then measured directly (Fig. 1). All hairs within and on the scribed line were epilated singly with forceps in the direction of hair growth, to minimize damage to the hair bulb. The collected hairs were transferred to a 10-mm wide, double-sided adhesive tape, mounted on a microscope slide, and aligned so that the roots were 10 to 15 mm away from the tape edge near the middle of the slide (Fig. 2). Hairs were examined microscopically using transmitted light at a magnification of 120, with a graticule eyepiece calibrated so that one division was equal to 6·25 μm. Each was classified according to the growth phase: anagen, catagen or telogen. Diameters were measured 8 to 15 mm from the hair root in the anagen phase, a position chosen to eliminate the effects of weathering on fibre diameter. For hair in the catagen

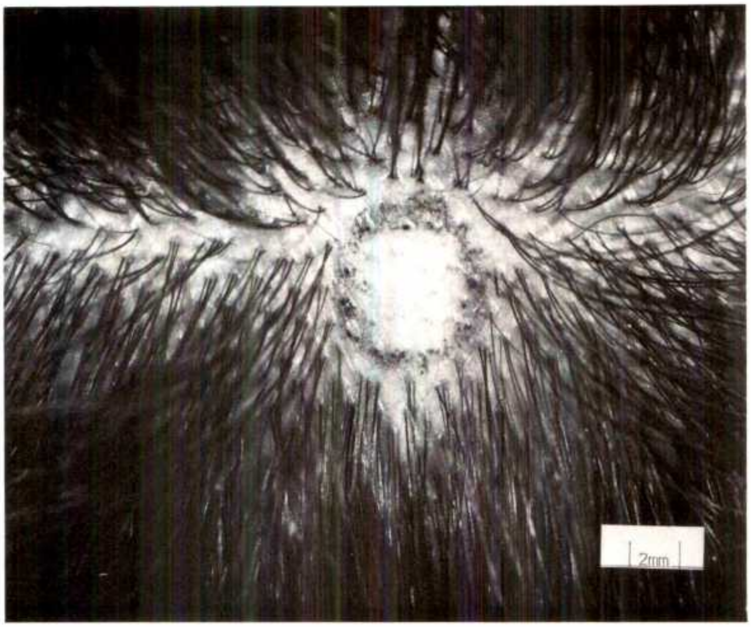

FIGURE 1. The defined limits of the unit area trichogram from the occipital site in a normal woman. All hairs within and on the line (an area of 35 mm^2) were epiliated.

and telogen phase, values were also taken at 35 mm from the root. Each hair was rotated through 180 degrees, and the largest (major axis) and smallest (minor axis) diameters measured. There were large variations between subjects in the major cross sectional axis as well as within subjects. The percentage difference between major and minor axes within subjects decreased with the diameter of the major axis. Typical differences ranged from 43% of the major axis (ie: minor 75 μm/major 132 μm) through to 12% (minor 44 μm/major 50 μm). Therefore, the diameter of each

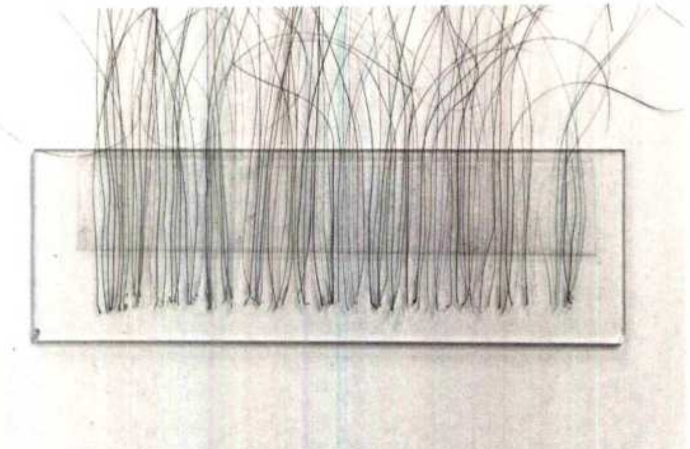

FIGURE 2. The epilated occipital hairs from a normal woman, dry mounted with double-sided adhesive tape attached to a microscope slide.

hair was assessed as the average of these two dimensions. It was this average which was used in calculating the mean diameters. Hairs were then removed from the slide, their lengths measured and the distal ends classified as: cut (undergone hair cutting), uncut (naturally pointed) or broken (weathered). All hairs were evaluated within 36 h of collection and a total of 7603 hairs were taken from the control group and patients (forty-five subjects) each providing two sampled sites, giving a mean of 85 hairs per site.

RESULTS

Hair density (Table 1)

In normal men, the hair densities of the frontal and occipital areas were not significantly different. This was true also in normal women, although normal men had greater hair densities in both frontal and occipital areas compared with normal women. In contrast frontal and occipital hair densities were diminished in both men and women with androgenic alopecia, with a greater reduction in density in both sites in the women.

Hair diameter (Tables 1 and 2; Figs 3 and 4)

The number of hairs greater than 40 μm cm^{-2} was not significantly different between the frontal and occipital areas in either normal men or women, although the total number in both sites was greater in normal men. The distribution of hair diameter compared with length in four normal women is shown in Fig. 3. However, there was a marked reduction in hairs greater than 40 μm in both men and women with androgenic alopecia. The frontal area was affected to a greater extent than the occipital area. In normal subjects it was found that in men only one hair in 428 in the frontal area had grown to 80 mm in length if its diameter was less than 40 μm. The ratio in the occipital area was 1:365 with a mean from both sites of 1:396. In men with androgenic alopecia none of the 778 hairs examined from the frontal area with a diameter of less than 40 μm grew to longer than 80 mm, while in the occipital area only 1 in 696 grew beyond 80 mm. In the females

TABLE 1. The unit area trichogram for normal males and females and patients with androgenic alopecia

	Normal				Androgenic alopecia			
	Male (n = 10)		Female (n = 10)		Male (n = 10)		Female (n = 15)	
	Frontal	Occipital	Frontal	Occipital	Frontal	Occipital	Frontal	Occipital
Hairs per cm^2 (density)	312 ± 15	311 ± 11	269 ± 12	271 ± 12	175 ± 6	219 ± 8	141 ± 7	189 ± 10
Range	256–393	261–356	234–345	233–352	148–212	185–272	69–186	140–273
Hairs per cm^2 > 40 μm in diameter (meaningful density)	286 ± 12	276 ± 10	241 ± 10	236 ± 12	121 ± 13	192 ± 5	107 ± 6	158 ± 10
Range	232–340	213–317	193–290	198–310	60 ± 167	176 ± 219	58 ± 146	113 ± 242
% Anagen phase hair	89·5 ± 0·8	91·9 ± 1·2	90·1 ± 1·1	90·7 ± 1·6	57·3 ± 5·5	85·4 ± 1·5	72·9 ± 2·3	82 ± 1·6
Range	84·6–93·9	86·8–97·8	85·6–95·7	84·2–97·0	33·3–75·9	80·9–90·9	56·8–86·5	67·4–91·5
% Telogen phase hair	9·1 ± 1	6·4 ± 1	8·8 ± 1	8·3 ± 1	37·3 ± 4	13·1 ± 2	23·4 ± 2	15·9 ± 1
Range	5·2–13·8	1·1–12·1	4·3–12·9	3·0–14·6	19·3–56·5	8·4–17·5	9·6–39·2	8·5–28·3
% Catagen phase hair	1·4 ± 0·2	1·7 ± 0·3	1·2 ± 0·3	1·0 ± 0·3	5·4 ± 1·0	1·5 ± 0·5	3·7 ± 0·7	2·1 ± 0·7
Range	0·8–2·1	0·0–3·1	0·0–2·7	0·0–2·7	2·4–10·6	0·0–3·4	0·0–10·7	0·0–7·7

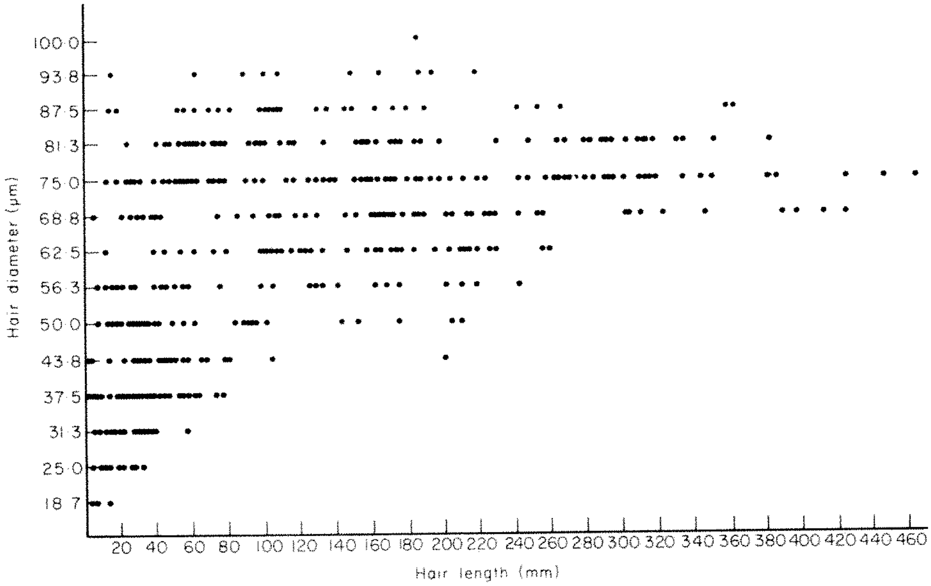

FIGURE 3. The distribution of hair diameter and corresponding length for uncut hairs from four normal females.

TABLE 2. Values obtained from normal and androgenic alopecia subjects

	Normal				Androgenic alopecia			
	Male n = 10		Female n = 10		Male n = 10		Female n = 15	
Total hairs sampled (7603)	Frontal	Occipital	Frontal	Occipital	Frontal	Occipital	Frontal	Occipital
Ratio of finding a hair < 40 μm in diameter and > 80 mm in length	1:428	1:365	1:322	1:410	0:778	1:696	1:249	1:193
	1:396		1:396		1:1474		1:221	
Longest hair (mm) < 40 μm in diameter per group	109	82	132	110	48	81	119	130
Range	22–109	21–82	14–132	60–110	11–48	4–81	15–119	18–130
Mean length (mm ± s.e.) of all hairs < 40 μm	26 ± 4	28 ± 2	24 ± 4	29 ± 3	10 ± 2	25 ± 4	17 ± 2	23 ± 3
Range	12–48	13–39	10–44	19–49	6–14	12–32	9–37	11–43
Mean group (% ± s.e.) Hairs < 40 μm in diameter per sample	8·5 ± 1	11 ± 1	10 ± 2	13 ± 2	34 ± 6	13 ± 2	27 ± 3	19 ± 3
Range	4·2–16·3	4·1–18·2	5·3–19·1	5·0–19·4	13–63	4–24	10–48	8–38
Mean hair diameter (μm ± s.e.)	68 ± 2	64 ± 2	70 ± 3	67 ± 2	54 ± 3	69 ± 3	60 ± 3	66 ± 4
Range	54–78	51–75	54–89	55–77	37–72	56–74	45–73	44–78

with androgenic alopecia, the probability of hairs in the frontal area less than 40 μm in diameter growing to greater than 80 mm was 1:249. In the occipital area of female patients with androgenic alopecia the ratio was found to be 1:193.

In normal subjects and patients with androgenic alopecia, the longest hair from any site with a diameter less than 40 μm had a length of only 132 mm, with only four hairs attaining a length greater than 100 mm. The length of hair was particularly reduced in the frontal area of the male patients in whom the longest hair measured was only 48 mm. The mean length of all hairs less than 40 μm in diameter varied between 24 mm (±4 s.e.) and 29 mm (±3 s.e.), except in the frontal area of the androgenic alopecia patients, when values of 10 mm (±2 s.e.) for men and 17 mm (±2 s.e.) for women were obtained.

In the frontal area of normal men, 8·5% (±1 s.e.) of hairs were less than 40 μm in diameter, compared with 34% (±6 s.e.) in men with androgenic alopecia. The corresponding occipital values were 11% (±1 s.e.) in normal men and 13% (±2 s.e.) in androgenic alopecia. In normal women only 10% (±2 s.e.) of hairs in the frontal area were less than 40 μm in diameter compared with 27% (±3 s.e.) in those with androgenic alopecia. In the occipital area, values were 13% (±2 s.e.) and 19% (±3 s.e.) in normal women and those with androgenic alopecia respectively. The increased percentage of hairs less than 40 μm in diameter in androgenic alopecia subjects, compared with the normal controls, is shown in Fig. 4. Although there was no significant difference between the mean hair diameters from the frontal or occipital areas in normal men and women, the mean diameters in the frontal area in the androgenic alopecia subjects were reduced.

Percentage of hair in growth phases: anagen, telogen, catagen (Table 1, Fig. 5)

The percentage of hairs in the anagen growth phase in normal subjects was similar in both sexes, and there was no significant difference in the frontal or occipital areas. In contrast, the

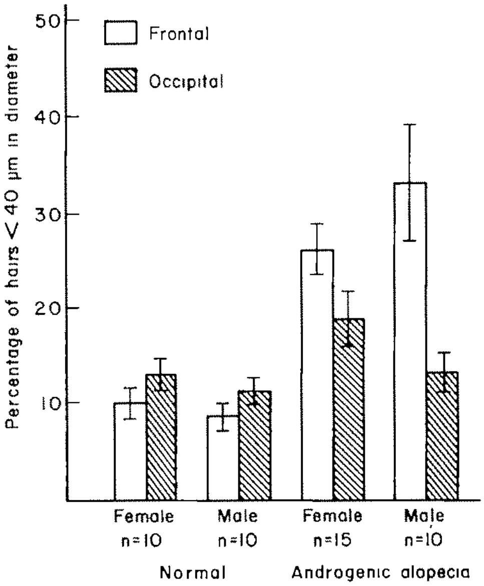

FIGURE 4. The percentage of hairs less than 40 μm in diameter in normal men and women compared with patients with androgenic alopecia, (mean ± s.e.).

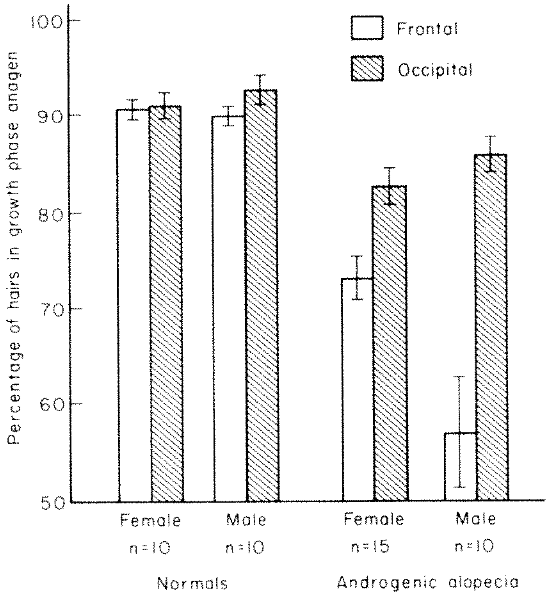

FIGURE 5. The percentage of hairs in the anagen growth phase in normal men and women compared with patients with androgenic alopecia, (mean ± s.e.).

percentage of hair in the anagen growth phase was markedly reduced in both men and women with androgenic alopecia, with the greatest reduction in the frontal area, especially in men (Fig. 5). The percentage of hairs in the telogen phase in normal men and women was not significantly different between the frontal and occipital areas. In the androgenic subjects the percentage was markedly increased in the frontal area, to a greater extent in the men, with an increase also in the occipital area in both sexes. The percentage of hairs in the catagen phase was not significantly different in either the frontal or occipital areas in either normal men or women, although these were increased in the frontal areas in androgenic patients of both sexes.

Statistical levels of significance were determined by the use of Student's t test.

DISCUSSION

In previous studies of the diameter of human scalp hair, in which direct measurements were made, both Jackson, Church & Ebling (1972), and Barman, Astore & Pecoraro (1965) obtained measurements in the same plane. The failure to allow for major and minor axis variation may explain the difference in our findings on mean diameters. We were unable to confirm the finding of Jackson *et al.* of two mean hair diameter peaks of 40 μm and 60 μm in females with diffuse thinning without detectable endocrine abnormality. These peaks may represent the mean major and minor axis values; however, the rather small number of hairs sampled ($n = 20$) per patient, which was further reduced for data evaluation by the exclusion of telogen phase hairs, makes interpretation of their results difficult.

The omission of a standardized pre-sampling procedure can result in distorted anagen:telogen ratios and may account for the differences between our results and those of Barman *et al.* (1965). They found an increase in telogen phase hairs in the entire scalp to be greater in normal men compared with normal women, an observation we could not confirm.

Although it is common to find an increased telogen:anagen ratio in androgen-dependent alopecias (Dawber, Sonnex & Ralfs, 1981; Tamm *et al.*, 1982) the use of the telogen count to indicate change has obvious limitations.

Our major objective was to define a relationship between fibre diameter and hair density as a measure of hair status. Our results for mean hair density (male: frontal 312, occipital 311; female: frontal 269, occipital 271) are higher than those of Barman *et al.* (1965) (222 cm^{-2}). In the latter study the number of fibres was determined optically without epilation from a 4-mm^2 area; such a small area might be expected to lead to sampling errors. Cottington, Kissinger & Tolgyesi (1977) measured the terminal hair density in women; a terminal hair was defined as being at least 2-cm long and of similar diameter to the long fibres. Their mean hair density value of 211 also underestimates the total number of hair fibres cm^{-2}. In particular, they were sceptical of the occurrence of hairs less than 40 μm in diameter since they found only one such hair in 4520 measured. In contrast, our data show that hairs less than 40 μm in diameter are widely distributed, but are unlikely to grow longer than 80 mm. Such hairs will not contribute to the appearance to the same degree as hairs having greater diameters (meaningful hair).

The change in the percentage of hairs less than 40 μm in diameter is of diagnostic significance in androgenic alopecia, where the conversion of meaningful hair (greater than 40 μm in diameter) to finer (less than 40 μm) is a predominant feature. In androgen-dependent alopecia, diameters are not the only measurements which change. There was also an increase in the number of resting (telogen/catagen phase) hairs. This is an important feature in the aesthetic appearance of the hair. A frequently observed characteristic of male androgenic alopecia was the occurrence of a large percentage of hairs of normal diameter, which were found to be of restricted growth. This characteristic is rarely seen in affected females, and as yet no satisfactory explanation of this presumably androgenic phenomenon is available.

General differences between male and female androgenic alopecia can be seen if the number of hairs less than 40 μm in diameter attaining a length greater than 80 mm is expressed relative to scalp area. In normal males the frequency of finding one hair less than 40 μm and greater than 80 mm was 1 per 1·4 cm^2 (frontal) and 1 per 1 2 cm^2 (occipital). In the normal female, the frequencies were 1 per 1·2 cm^2 and 1 per 1·5 cm^2 respectively. In male androgenic patients the frequency was 1 per 4·5 cm^2 (frontal) and 1 per 3·2 cm^2 (occipital) while in female androgenics, such hairs occurred in 1 per 1·8 cm^2 (frontal) and 1 per 1·1 cm^2 (occipital). Therefore, in males, the most severely affected sites have finer, shorter hairs. The number of hairs less than 40 μm in diameter is significantly increased ($P < 0{\cdot}0001$), and the percentage of hair in the growth phase (anagen) is significantly reduced, while that in telogen is increased ($P < 0{\cdot}0001$). Consequently, the chance of finding a hair less than 40 μm, and longer than 80 mm was decreased compared with normal men. In contrast, in female androgenics the frequency was closer to that of normal females, i.e. although the female androgenics had fewer hairs per cm^2, the hair that remained resembled that found in normal women.

We conclude that the accurate determination of hair density, meaningful density, hair diameter and identification of the hair growth phases requires the removal of hair from a measured site on the scalp following a standardized pre-sampling washing procedure. It is proposed that the wider application of this 'unit area trichogram' will provide a reliable assessment of basal hair status and a clinically acceptable technique to monitor the response of hair to therapeutic agents.

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Pharmacology and Treatment

Retinoid concentrations in skin, serum and adipose tissue of patients treated with etretinate*

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SUMMARY

Synthetic and natural retinoids were analysed in epidermis, dermis, subcutis and serum of twenty-seven patients treated with etretinate (0·6–1·0 mg/kg/day) for 1–36 months. The concentrations of etretinate (including its major metabolite) in serum and normal-appearing epidermis were 150–600 ng/ml and 50–350 ng/g, respectively. The serum and epidermal values were significantly correlated ($P < 0{\cdot}05$). The drug progressively accumulated in the subcutis attaining a maximum value of 15,500 ng/g. After the treatment, etretinate disappeared from the epidermis within 1 week. By contrast, the drug remained in the subcutis for several months after cessation of treatment. The epidermal composition of endogenous retinoids changed during etretinate therapy as reflected in an increased ratio of 3-dehydroretinol to all-*trans* retinol.

Synthetic retinoids should ideally exert therapeutic activity without accumulating in the organism and without causing serious side-effects. Etretinate (Ro 10-9359), the ethyl ester of an aromatic analogue of retinoic acid, has a favourable therapeutic index and is active in a number of skin disorders, some of which were previously resistant to all treatments (Bollag & Hanck, 1979; Orfanos 1980).

Unfortunately, during prolonged treatment, etretinate accumulates in the body and its plasma half-life increases to at least 100 days (Paravicini, 1981). This is of special concern in view of the teratogenicity of the drug (Hummler & Schüpbach, 1981). The storage compartment for etretinate has not been identified but the liver has been implicated by analogy with the storage of vitamin A (Paravicini, 1981). Etretinate, however, is less lipophilic than the fatty acid esters of vitamin A stored in the liver. Also, the transport of etretinate and vitamin A differ; whereas the former is bound to plasma lipoproteins and albumin, it does not bind to the retinol-binding protein (RBP) which releases vitamin A from the liver (Vahlquist *et al.*, 1981).

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* A preliminary report was presented at the Sixteenth International Congress of Dermatology, Tokyo, May 1982. Correspondence: Dr A.Vahlquist.

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From studies on patients treated with etretinate, the major metabolite in plasma (Ro 10-1670) has been identified (Hänni, 1978). This metabolite, a free carboxylic acid, is probably more active than etretinate at the target level (Aoyagi *et al.*, 1981). Ro 10-1670 binds to cellular retinoic acid binding protein, and a number of its biological functions are similar to those of retinoic acid (Jetten & Jetten, 1979; Jetten, 1981). It is, therefore, possible that etretinate therapy might interfere with cellular vitamin A metabolism.

We have previously shown that hydrolysed extracts of human epidermis contain two preponderant forms of vitamin A, retinol (A_1) and 3-dehydroretinol (A_2), detectable by high-performance liquid chromatography (HPLC) (Vahlquist, 1982). Synthetic retinoids may also be analysed by this technique. In this study, we have examined the distribution of etretinate in the skin and its possible effects on the vitamin A composition of the skin.

METHODS

Patients and treatment

We studied twenty-seven patients (eleven women, sixteen men) with the following diagnoses: psoriasis vulgaris (10), pustular psoriasis (4), Darier's disease (5), tylotic hand eczema (3), ichthyosis vulgaris (2), ichthyosiform erythroderma (2) and multiple basal cell carcinoma (1). Three categories of patients were studied (Table 1). In one group of patients a cross-sectional study was undertaken, in a second the patients were observed longitudinally and in a third the patients were studied during the wash-out period only. No systemic drug other than etretinate (Ro 10-9359, Tigason®) was allowed, except for one patient who was being treated with methotrexate and prednisolone. Topical therapy included corticosteroids, dithranol and emulsifying ointment. In the longitudinal study no topical therapy at all was allowed.

TABLE 1. Data on the different categories of etretinate-treated patients

	Study design		
	Cross-sectional	Longitudinal	Wash-out
Number of patients	18	8	11[a]
Age (years)	43 (12–61)[b]	53 (23–73)	40 (12–64)
Duration of therapy (months)	12 (2–40)	1	11 (2–45)
Daily dose (mg)	20–50	50	20–50
Accumulated dose (g)	10·0 (1–32)	1·5	7·8 (2–27)
Max. time of follow-up after withdrawal of therapy (months)	—	2	24

[a] Ten of the patients were also included in the cross-sectional study while on therapy.

[b] Mean and range.

Skin biopsies

Superficial skin (10–20 mg) of normal appearance was obtained from the buttocks by shave biopsy as described elsewhere (Vahlquist *et al.*, 1982b). The specimens are referred to as 'epidermis' in the text although they contained small amounts of papillary dermis. From the underlying dermis, a 3-mm punch biopsy (2–5 mg) was taken. Small amounts of subcutis contaminating these biopsies were rapidly removed under a dissection microscope. Specimens of lower subcutis (5–15 mg) were collected through the punched hole using a biopsy forceps (Strümpel No. 12085, Stille). The samples were blotted to remove blood, immediately frozen on dry ice and stored at −70°C until processed.

Serum

Blood was collected at the occasion of biopsy. In the longitudinal study fasting blood was drawn 11–16 h after a dose of etretinate.

Reagents. The retinoids were gifts from Hoffman-La Roche, Basle. The sources of the other materials have been described earlier (Vahlquist, 1982; Vahlquist *et al.*, 1982b).

Analysis. A previously described technique with minor modifications was used (Vahlquist, 1982). Internal standards (Ro 12-0586 and all-*trans* retinoic acid) were added. Tissue specimens (2–20 mg) and serum (150–500 μl), were hydrolysed in KOH-ethanol whereby etretinate (Ro 10-9359) was completely converted to Ro 10-1670. The neutral and acidic retinoids were separated by adjusting the pH before extraction with hexane. The extracts were subjected to reversed-phase HPLC. Control experiments established that neither of the internal standards co-chromatographed with substances present in the endogenous samples. The detection limit for Ro 10-1670 was 10 ng/ml in serum, 15 ng/g in epidermis and dermis and 100 ng/g in subcutis using the prescribed sample quantities.

Most tissue samples require hydrolysis in order to extract the retinoids quantitatively. In a few experiments an alternative approach was used whereby, in order to distinguish Ro 10-9359 and Ro 10-1670, the hydrolysis was omitted. Specimens (200 mg) of subcutis and epidermis were obtained from axillary skin during plastic surgery of a patient who had been treated with etretinate for several months. The samples were extensively homogenized, freeze-dried and extracted with 5 ml of methanol by vigorous shaking for 10 min. After centrifugation, aliquots of the clear supernatant were analysed by HPLC as described above. The areas of the peaks appearing at the positions of Ro 10-1670 and Ro 10-9359, respectively, were compared. Blank tests with skin samples from untreated patients were included as a control.

Statistics. The statistical analysis was by Student's t test using paired values where possible.

RESULTS

Cross-sectional study

Table 2 summarizes the results obtained in patients receiving 20–50 mg of etretinate daily for various periods of time. Although the values varied substantially from one patient to the other it can be seen that the mean concentrations of the drug and retinol in epidermis and serum are similar. The accumulation in subcutis is, however, more pronounced for etretinate. The highest concentration in subcutis (15,500 ng/g) was found in a woman (wt. 60 kg) who had received over 36 months a cumulative dose of 31·5 g.

3-Dehydroretinol occurred in the patients' epidermis but was undetectable in serum and subcutis. The mean molar ratio between 3-dehydroretinol and retinol was 0·48 during treatment as compared with a normal value of 0·21 (Vahlquist *et al.*, 1982b).

In order to investigate the distribution of retinoids in esterified and non-esterified species, a semiquantitative analysis of samples from one patient receiving etretinate (see Methods) was undertaken. In this analysis, the hydrolysis step was omitted. This showed that Ro 10-9359 (the ethyl ester of Ro 10-1670) and retinyl esters account for over 95% of the total amount of retinoids in subcutis. By contrast, the epidermis contains twice as much Ro 10-1670 as Ro 10-9359. By comparison 65% of the retinol and 90% of the 3-dehydroretinol are esterified in epidermis. In serum, Ro 10-1670 and Ro 10-9359 often occur in about equal concentrations whereas retinol predominates over the retinyl esters by a factor of ten (Vahlquist *et al.*, 1981).

Since the retrospective data suggested that etretinate is stored in subcutis and that etretinate therapy interferes with the vitamin A composition of normal appearing epidermis, a prospective study was performed to gain more detailed information.

Longitudinal study

Fig. 1 is a logarithmic representation of the etretinate levels in uninvolved epidermis, dermis, subcutis and serum of eight patients studied before, during and after a 1-month treatment. Already after the first week of therapy the etretinate concentration in subcutis exceeds that of the other tissues. During the following 3 weeks the drug continues to accumulate in subcutis but not in serum and epidermis. After the treatment is suspended, there is a rapid clearance of etretinate from epidermis, a less rapid clearance from dermis and serum, and virtually no clearance at all from subcutis for the first 7 weeks following withdrawal of the drug. All patients showed the same pattern and the interindividual ranges of values were small. The dermal values may be somewhat overestimated since contamination of the biopsies by etretinate-rich adipocytes cannot be excluded. The serum and epidermal concentrations of etretinate were probably significantly correlated ($r = 0{\cdot}54$; $P < 0{\cdot}05$).

TABLE 2 Skin and serum concentrations of etretinate and vitamins A during etretinate therapy (cross-sectional data). Details of the treatment are given in Table 1

Specimen	Number of patients	Etretinate[a]	Retinol (A_1)	3-Dehydroretinol (A_2)
Epidermis (ng/g wet wt)	18	227 (30)[b]	274 (25)	132 (31)
Subcutis (ng/g wet wt)	13	7512 (1228)	1850 (318)	ND[c]
Serum (ng/ml)	13	493 (84)	256 (13)	ND

[a] The sum of etretinate and its main metabolite, expressed in equivalents of Ro 10-1670 (see Methods).

[b] Mean (S.E.M.); to convert to nmoles/g (or ml), the values should be multiplied by 0·00350 (retinol), 0·00352 (dehydroretinol) and 0·00307 (etretinate).

[c] Not detectable.

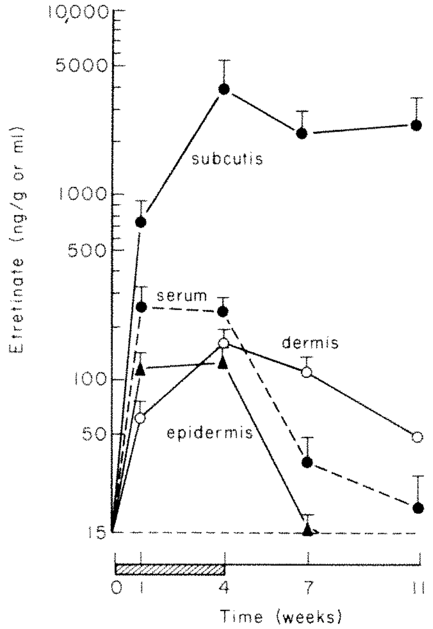

FIGURE 1. Tissue (——) and serum (- - - -) mean concentrations of etretinate including its main metabolite (longitudinal study). Values expressed in equivalents of Ro 10-1670. The detection limit for the drug in epidermis is indicated by the horizontal line. The bars represent the standard errors of the means. The hatched area represents etretinate therapy.

Table 3 shows the results of the vitamin A analysis in the longitudinal study. Serum values are excluded as they have previously been shown to be unaffected by etretinate therapy (Lauharanta, 1981; Vahlquist *et al.*, 1981). The retinol concentrations in epidermis and subcutis decrease temporarily during therapy but the changes are not statistically significant. The 3-dehydroretinol concentration in epidermis increases during and after etretinate treatment but in our limited series, the difference compared to the pre-treatment values is not statistically significant. However, the ratio of 3-dehydroretinol to retinol changes significantly during treatment with a mean increase of 96% ($P < 0\cdot01$). At 3–7 weeks post-treatment the ratio is still elevated ($P < 0\cdot05$) compared to the initial value. The patients were not followed long enough to observe a normalization of the ratio.

Wash-out of etretinate

The slow elimination of etretinate from subcutis observed in the longitudinal study prompted an extended investigation of the wash-out phase. Fig. 2 shows the drug concentrations in subcutis and serum of twelve patients withdrawn from therapy for 1–24 months. During the first 10 months after cessation of therapy the values in subcutis seem to conform to an exponential decay with a $T_{\frac{1}{2}}$ of about 3 months. Etretinate was detectable in the subcutis of one patient studied 18 months after discontinuation of the drug. Six months later, however, the drug was undetectable in another patient. The serum values were at a much lower level and the drug was undetectable in sera obtained 10 months or more after cessation of therapy.

Other investigations. Skin surface lipids were collected (Vahlquist *et al.*, 1982b) from the

TABLE 3. Variations in the vitamin A concentrations of the skin in relation to etretinate therapy

		Relation to therapy[b]		
	No.[a]	Before	During	After
Epidermis				
Retinol (A_1)	5	243 (42)[c]	180 (23)	221 (19)
3-dehydroretinol (A_2)	5	57 (11)	65 (13)	113 (34)
A_2/A_1 (molar ratio)	5	0·25 (0·02)	0·49** (0·04)	0·53* (0·12)
Subcutis				
Retinol	5	2020 (514)	1860 (171)	2066 (184)

[a] Number of patients in whom both retinol and 3-dehydroretinol were analysed at all three occasions.

[b] Values during therapy were obtained within 1–4 weeks of treatment and values after therapy were obtained 3–7 weeks following withdrawal of the drug.

[c] Mean concentrations (s.e.m.) in ng/g wet weight

* $P < 0·05$, and ** $P < 0·01$, as compared with value before therapy.

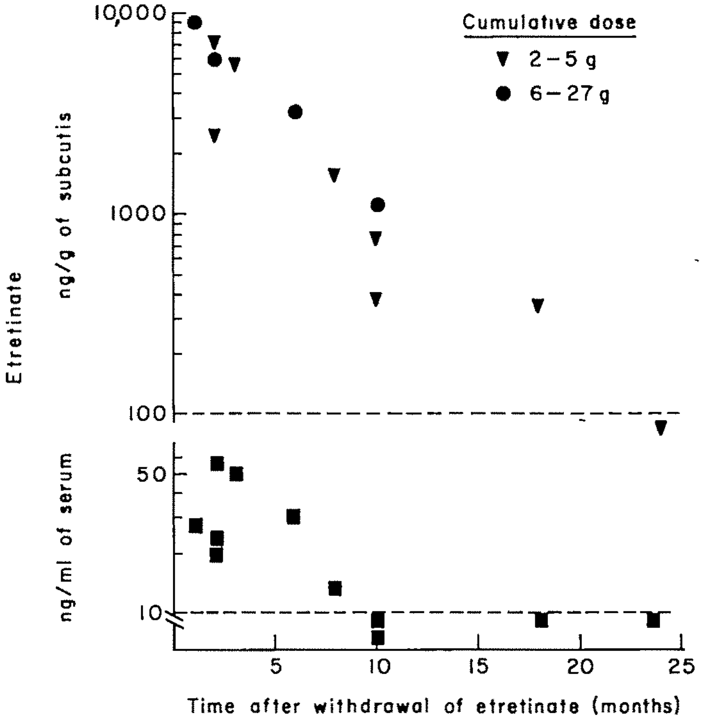

FIGURE 2. Scatter plot of the etretinate concentrations in subcutis (● or ▼) and serum (■) as a function of time after withdrawal of treatment (wash-out study). Values expressed in equivalents of Ro 10-1670. The horizontal lines indicate the detection limits for subcutis and serum respectively

forehead of a patient treated with 50 mg of etretinate daily for 1 week. Repeated analyses revealed that, whereas retinol was found in normal amounts (Vahlquist *et al.*, 1982b), the etretinate concentration was below the detection limit of the assay.

In two patients treated with 25 mg and 50 mg of etretinate daily, epidermal retinoids were analysed before and 10 min after whole-body UV-irradiation (380–320 nm: 1·9 mW/cm^2; 320–300 nm: 2·6 mW/cm^2). The etretinate concentrations before (94 and 274 ng/g wet weight) and after irradiation (86 and 365 ng/g) were similar. By contrast, the retinol concentrations were reduced by 60–70% after the irradiation, confirming previous observations (Berne *et al.*, to be published).

DISCUSSION

Our results clearly identify adipose tissue as a storage site for etretinate in man. The drug is stored in the adipocytes almost exclusively in its original ester form (Ro 10-9359). The subcutaneous drug concentrations increase rapidly during the first month of therapy reaching values of 2–10 μg/g which are 10–30 times higher than those observed in dermis and epidermis (Fig. 1). Apparently, the adipocytes later become saturated since the values of etretinate in subcutis did not exceed 16 μg/g despite the fact that some of the patients had been on treatment for several years (Table 1). By assuming an adult fat mass of 10 kg and a uniform distribution of the drug in the tissue, it can be calculated that adipocytes would store about 100 mg of etretinate after a few months of therapy. This represents only a small percentage of the administered dose. Etretinate apparently disappears only slowly from subcutis since it can be detected even 18 months after withdrawal of therapy (Fig. 2). It is plausible therefore that the storage of etretinate in adipose tissue may explain the slow plasma clearance of the drug (Paravicini, 1981). However, other 'deep' compartments, such as the liver, must also be considered.

What are the practical implications of these findings? First, it is reasonable to assume that obese patients suffer a greater risk of accumulating etretinate although this has not been investigated in our series of patients. Second, etretinate may compete with lipids and other drugs for storage sites in the adipocytes. Speculatively, compounds that are able to displace etretinate from these storage sites may exist.

In sharp contrast to the subcutis, the epidermis does not accumulate etretinate progressively (Fig. 1). Furthermore, after therapy a rapid disappearance is observed which coincides with the recession of cutaneous side-effects and, all too often, relapse of the skin disease. The epidermal concentrations of the drug exceeded those in the dermis during the initial stage of therapy (Fig. 1). Our results imply an active uptake of etretinate by the epidermal cells. No evidence of a sebum-mediated transport of the drug to the epidermis was found.

Epidermis, like serum (Paravicini, 1981; Vahlquist *et al.*, 1981) and blister fluid (Lauharanta & Paravicini, 1982) from etretinate-treated patients contains both Ro 10-9359 and Ro 10-1670. This information can be gained only by omitting hydrolysis from the analytical procedure. The unequal distributions of Ro 10-1670 and Ro 10-9359 in serum and epidermis (with relatively larger amounts of Ro 10-1670 in epidermis), may be due to the facts that (1) albumin (carrying Ro 10-1670) occurs in the interstitial fluid at relatively higher concentrations than the lipoproteins (carrying Ro 10-9359) (Vermeer, Reman & van Gent, 1979) and (2) that keratinocytes are able to produce Ro 10-1670 by de-esterifying Ro 10-9359 (Kitano, Okada & Nagase 1982).

Ro 10-1670 is structurally closely related to endogenous retinoic acid. The latter compound, which is difficult to detect in the skin (Vahlquist, 1982), was not investigated in the present study. Retinol, presumably the precursor of retinoic acid in epidermis was, however, studied. The concentrations of retinol and etretinate in epidermis are somewhat similar (Table 2). Interestingly, the response of the two retinoids to UV-irradiation differs. While the retinol concentration in epidermis decreases markedly after UV therapy the etretinate level seems to be unaffected. 3-Dehydroretinol, the other endogenous retinoid studied, occurs predominantly in the epidermis (Table 2). This compound is of yet unknown origin but is probably an epidermal metabolite of retinol (Vahlquist *et al.*, 1982b). Changes in the composition of epidermal vitamin A are reflected in the ratio between 3-dehydroretinol and retinol. The ratio is increased in disorders characterized by hyperproliferative epidermis (Vahlquist, 1980; Rollman & Vahlquist, 1981; Vahlquist *et al.*, 1982a). Etretinate therapy, which is known to induce the proliferation of normal epidermis (Fritsch *et al.*, 1981), was found to raise the 3-dehydroretinol/retinol ratio to twice its original value (Table 3). It is uncertain whether this change is due to the direct interference of etretinate with epidermal vitamin A metabolism or whether it is an indirect effect, but further studies may shed light on these matters.

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Treatment of keratoacanthoma with intralesional bleomycin

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SUMMARY

Six patients with keratoacanthoma were successfully treated with intralesional injection of bleomycin. This new therapy produced a marked shortening of the clinical course without any complications.

Keratoacanthoma is a common, self-healing epithelioma of the skin occurring mainly on the exposed areas of elderly people. It usually reaches its full size within several weeks and involutes spontaneously within several months, generally in less than 6 months, although the time is variable.

Various therapeutic modalities which have been reported to be successful include topical application of demecolcine (Belisario, 1965), intramuscular injection of methotrexate (Kestel & Blair, 1973), curretage (Reymann, 1977), irradiation (Farina *et al.*, 1977), intralesional injection of fluorouracil (Odom & Goette, 1978), intralesional injection of neocarzinostatin (Kinoshita, 1979), oral administration of 13-*cis*-retinoic acid (Haydey *et al.*, 1980), intralesional injection of interferon (Ueno *et al.*, 1981) and topical application of fluorouracil (Goette *et al.*, 1982).

Bleomycin is an antineoplastic agent isolated from *Streptomyces verticillus* and has been used mainly for epithelial carcinoma. Recently its diluted solution has been widely used in Japan to treat verruca vulgaris and its usefulness has been reported from other countries (Bremner, 1976; Bakker, 1982). We now report that intralesional injection of bleomycin is another simple and effective measure for the treatment of keratoacanthoma.

METHODS

Two men and four women (aged 52 to 79 years) with keratoacanthoma were treated with intralesional injection of bleomycin. We used bleomycin dissolved in saline at a concentration of

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1 mg/ml, which was further diluted with an equal amount of 0·5% lignocaine to relieve the pain induced by bleomycin. An appropriate amount of the solution, ranging from 0·2 to 0·4 mg according to the size of the lesions, was injected intralesionally through a No. 27 needle. A small biopsy specimen was taken from one of the edges to confirm the clinical diagnosis, but the biopsy was not large enough to alter the clinical course. The patients were seen every few weeks to make sure the lesion was disappearing.

CASE REPORTS

Case 1. A woman aged 69 had noticed a gradually enlarging, crateriform tumour on the ulnar surface of her left forearm 3 months before the first consultation. The size of the tumour was 19 × 16 mm (Fig. 1a); 0·4 mg of bleomycin was injected into the lesion. One week later, a large part of the tumour dropped off, leaving a raised peripheral border. She was followed up for the subsequent 5 weeks without any more treatment, when the persistent peripheral border became flattened (Fig. 1b). Three months later, on her last visit, the affected site was only slightly erythematous with no sign of recurrence (Fig. 1c).

Case 2. A 53-year-old man was seen with a tumour (10 × 10 mm) on his right cheek of 1 week's duration. Bleomycin (0·2 mg) was injected into the lesion. Three weeks later, at his next visit, the lesion had already disappeared completely.

Case 3. A 56-year-old woman had had a verrucous tumour (7 × 5 mm) on the right side of the dorsum nasi for the past 2 months. One week after 0·2 mg of bleomycin had been injected intralesionally, only the upper part of the tumour had dropped off, leaving a papillomatous plaque on the site, which became crusty 2 weeks later. A second injection of the same dose of bleomycin was repeated at this time. Two weeks later, the crust fell off, leaving a pigmented area.

Case 4. A 52-year-old woman consulted us for a tumour (10 × 10 mm) on the root of her nose which had developed in the past 2 weeks. Intralesional injection of 0·2 mg of bleomycin was carried out at weekly intervals. One week after the second injection, the tumour markedly decreased in size and completely regressed after 2 more weeks.

Case 5. A woman aged 79 was seen with a tumour (15 × 12 mm) on her left cheek of 2 months' duration. One week after 0·4 mg of bleomycin had been injected into the lesion, the lesion became fluctuant and on removal of the central horny plug a whitish necrotic mass was discharged, leaving an elevated peripheral border. One more injection of the same dose of bleomycin induced a complete flattening of the peripheral border 1 week later.

Case 6. A man aged 54 had noticed a small tumour (8 × 8 mm) at the mucocutaneous border of the upper lip 1 month before the consultation. One-tenth mg of bleomycin was injected intralesionally. In 1 week a major part of the lesion dropped off and in the following 3 weeks the lesion cleared completely without scarring.

DISCUSSION

Intralesional injection of a diluted solution of bleomycin has proved to be a simple and effective way to eliminate keratoacanthoma promptly. Since it is diluted with an equal amount of 0·5%

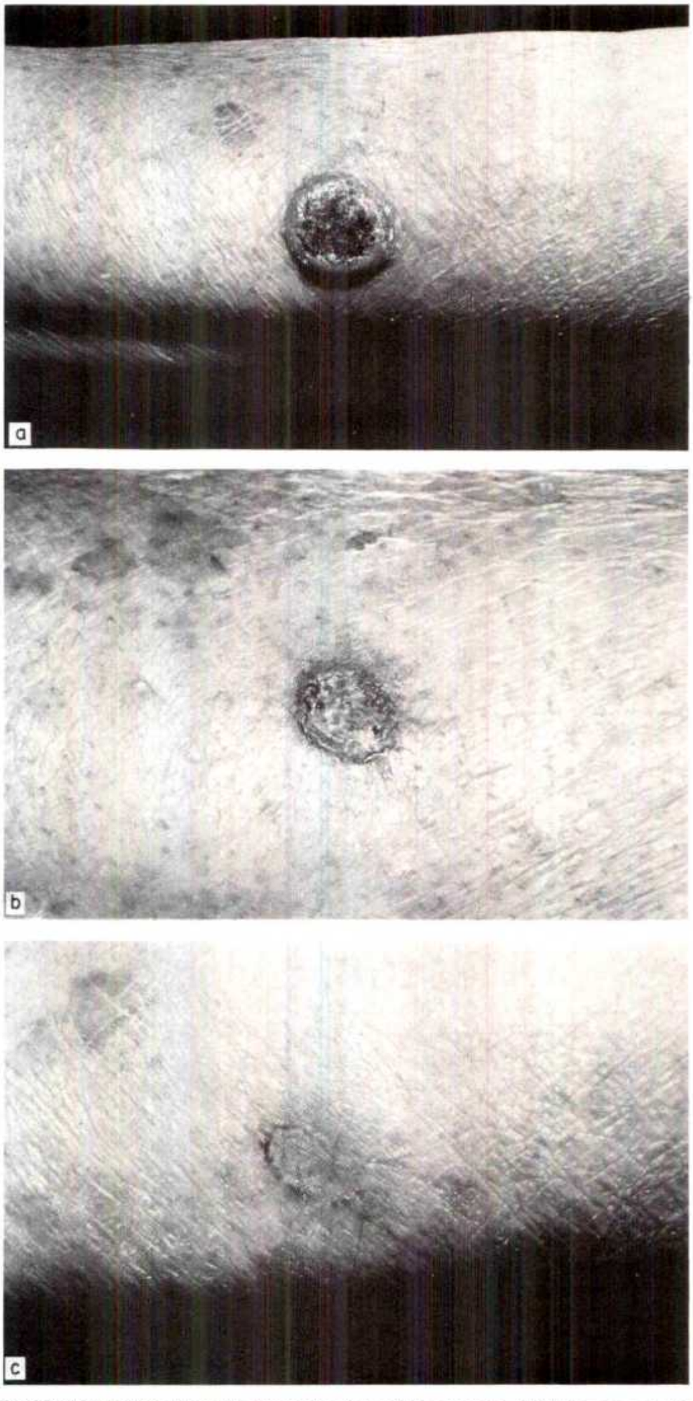

FIGURE 1. (a) Typical keratoacanthoma before injection of bleomycin. (b) A large part of the tumour has dropped off after one injection. (c) Only an erythematous patch is seen after 3 months.

lignocaine, we could obtain small biopsy specimens subsequently without any further anaesthesia. Of course, there is no need to perform such a biopsy in every case, particularly in those with the typical clinical features of a firm hemispherical nodule with crateriform keratotic plug, sharply marginated raised border and undamaged surrounding skin.

Although the diagnosis can be made from its characteristic appearance and clinical course, some clinicians recommend total excision of the lesion for fear that it is in reality a squamous cell carcinoma. However, since keratoacanthoma itself is a self-healing disorder, it is desirable not to leave an operative scar following the treatment of this disease. The intralesional injection of bleomycin leaves no unsightly scar and the cosmetic results are good.

The time required to eliminate the lesion, as determined by its complete flattening, ranged from 2 to 6 weeks. This is comparable to the results reported with intralesional injection of fluorouracil (Odom & Goette, 1978), although we did not carry out weekly injections in every case as with intralesional fluorouracil injection. The required time was apparently independent of the duration of the lesions, for instance in case 2 it was 1 week, while in case 1 it was 3 months.

As a rule, a larger part of the tumour drops off after one injection in a few weeks, although a peripheral border or papillomatous surface may persist in large lesions. Thus, to determine whether bleomycin is efficacious or not, it is sufficient to follow up the patients for only a few weeks. This reduces the hazard of a marked delay in beginning the treatment in the case of squamous cell carcinoma.

We thus regard intralesional bleomycin as one of the first choices for the therapy of keratoacanthoma.

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Case Reports

Cryoglobulinaemia and angiomatosis*

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SUMMARY

A 61-year-old woman is presented with essential cryoglobulinaemia and a 25-year history of recurrent ulceration of the lower legs. She developed multiple benign angiomatous nodules on the face, earlobes and neck. Histologically, they simulated an angiosarcoma and are therefore of considerable diagnostic importance.

CASE REPORT

Mrs I.J. first developed ulceration of the anterior lower legs in 1957 after minor trauma; since then, the ulcers have recurred periodically with exacerbations, usually during the winter months. She has had numerous admissions to hospital over the years, on some occasions requiring several months of in-patient treatment. Previous skin biopsies had shown changes of an allergic or leucocytoclastic vasculitis and extensive investigations have failed to reveal the cause of the vasculitis. She has been on long-term oral steroids which, in retrospect, have not influenced the course of her disease. The lesions did not respond to dapsone or azathioprine, but have always healed with rest in hospital. Her general health has been otherwise good, apart from angina, mild arthritis and steroid-induced diabetes.

She was admitted to Wycombe General Hospital in 1978 because of extensive ulceration of the lower legs and the recent occurrence of vascular papules on the face and neck.

On examination, she was rather an obese woman with marked reticulate, erythematous lesions on her face (Fig. 1) and upper arms; discrete, small, soft, nodular lesions on her face, earlobes and neck, some of which were haemorrhagic and others flesh-coloured; and pyoderma gangrenosum-like ulceration of the lower legs. In the course of cleaning some of her necrotic lesions, a large basal cell carcinoma was revealed on the forehead and another in front of the left ear. These were excised and skin-grafted.

During this admission she was given a transfusion of packed red cells, which caused an immediate and dramatic flare-up of the vasculitic lesions, especially on the upper arms and cheeks. This was so severe that the transfusion had to be discontinued. In the light of a negative

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* This case was presented at the Royal Society of Medicine (Section of Dermatology) on 21 October 1982.

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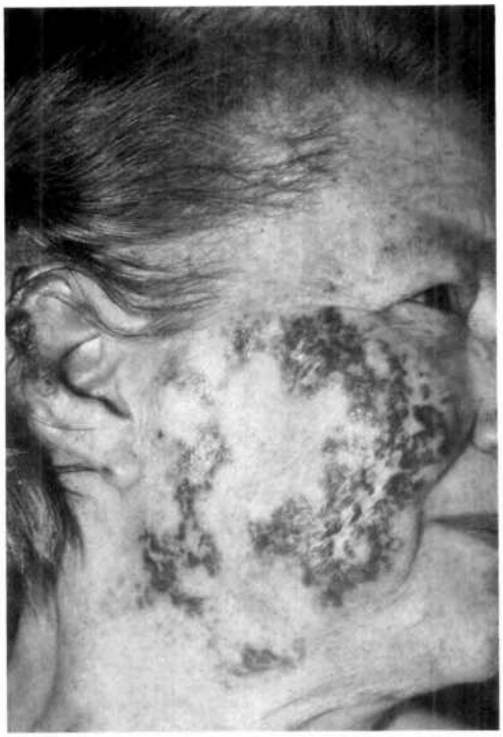

FIGURE 1. Reticulate vasculitic lesion on the face with necrosis of the outer margin of the pinna.

Coombs' test and the absence of atypical antibodies, it was felt that this exacerbation must have been provoked by the low temperature of the transfusion.

She stayed in hospital for about 6 months until the ulceration had healed completely. During the past 4 years she has remained fairly well, although she continues to develop ulcers on the legs, which are slow to heal and confine her to home for many months at a time. In November 1981, she was admitted to Westminster Hospital for further investigation and presented with extensive scarring from old healed lesions.

Investigations

A full blood count showed a normochromic, normocytic anaemia (haemoglobin 9·8 g/l on admission), with a persistent neutrophilia (total white count ranging from $13{\cdot}1$–$27{\cdot}7 \times 10^9$/l). The erythrocyte sedimentation rate fluctuated between 30 and 118 mm/h. The following investigations were all found to be normal or negative: clotting screen and platelet count, bone marrow aspirate and trephine, urea and electrolytes, creatinine clearance, liver function tests, auto-antibody screen, skeletal survey and a CAT scan of the chest and abdomen.

Cryoglobulin studies

She was found to have an IgG kappa cryoglobulin present in her serum at a concentration of 4 g/l at 37°C and 2 g/l at 24°C. The IgG paraprotein was completely precipitated at 4°C and 50% precipitated at room temperature. No Bence–Jones protein was detected in the urine. The immunoglobulins were otherwise normal. Complement (C3 and C4) were both slightly raised, but no C3 conversion products were detected.

Immunological investigations

Her peripheral blood lymphocyte count showed a lymphocytosis. T cells (by the standard E-rosette test) were 36% (2681/mm) (normal range 470–1800). B cells (bearing IgA, IgG or IgM) were 2% (149/mm) (normal range 100–1000). There was no evidence of cellular immunodeficiency, but an apparent imbalance of lymphocyte subpopulations. OKT4 (Helper T) cells were 33% (normal mean 46%); OKT8 (suppressor/cytotoxic T) cells were 30% (normal mean 25%). The helper/suppressor ratio of 1/1 (normal ratio 1/8) showed evidence of 'oversuppression', consistent with the abnormally low B-cell percentage.

Lymphocyte function studies (PHA, MLR and Candida killing) were all normal.

Polymorph function tests (staphlococcal inhibition and Candida killing) were also found to be normal.

No complexes were detected in the cryoprecipitate.

Viscosity studies

In November 1981, her viscosity studies were normal. In July 1982, at the time of an acute exacerbation of her skin condition, a marked increase in plasma viscosity was noted, with an increased temperature dependence. Two-dimensional immunoelectrophoresis showed a large increase in both fibrinogen and fibronectin.

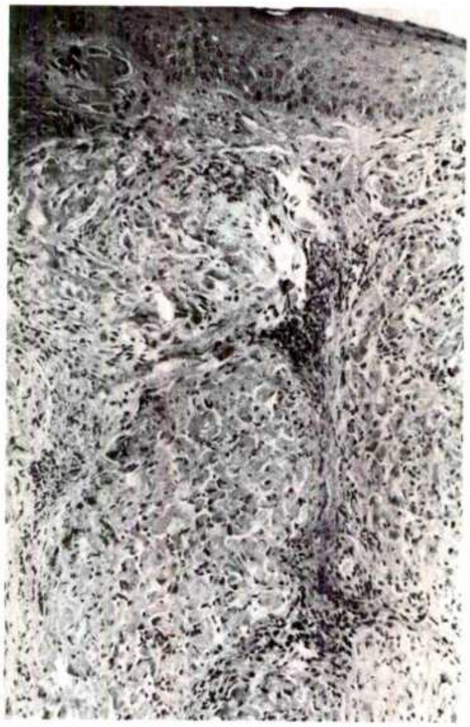

FIGURE 2. Histology of an angiomatous nodule (low power) showing the dermis occupied by nodular masses of anastomosing vascular channels, (H & E).

Histology and immunofluorescence

A nodule on the left ear and a clinically similar lesion on the left side of the neck both showed an intact epidermis with the dermis completely occupied by nodular masses of anastomosing vascular channels lined by endothelial cells and containing deposits of dense eosinophilic amorphous material (Figs 2 and 3). The endothelial cells varied in size and shape with irregular nuclei. Mitoses could not be seen, but there were ill-defined areas of necrosis. A remarkable feature was the presence of apparent collagen fibre dissection by the endothelial cells. A few extra-vascular deposits were present but there was no foreign body reaction to these and inflammatory changes were minimal.

The proliferating blood vessels were arranged in closely packed nodules surrounded by bands of connective tissue giving a jigsaw-like pattern, and these nodules extended into the subcutaneous tissues. Normal appendages were absent. Elastic stains showed no evidence of any larger blood vessel surrounding any part of the vascular proliferation.

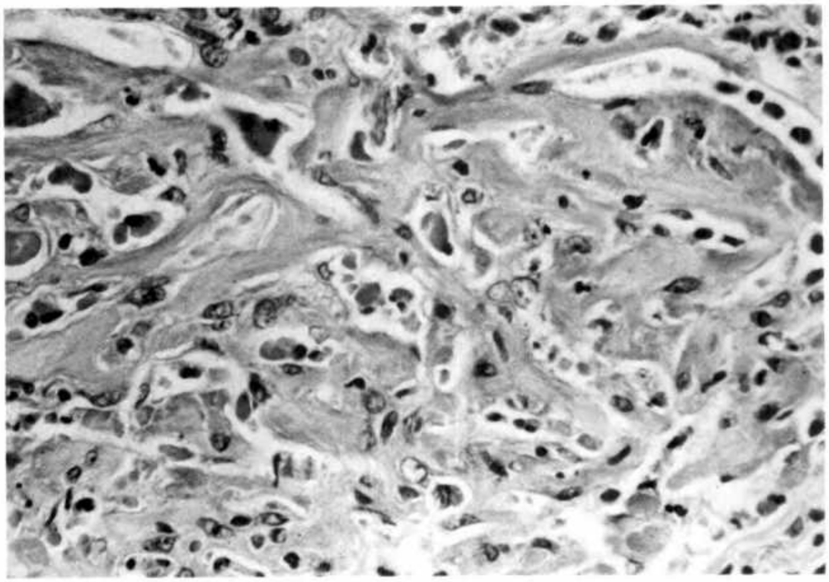

FIGURE 3. Histology of an angiomatous nodule (high power) showing the vascular channels lined by endothelial cells and containing deposits of dense eosinophilic amorphous material (cryoprecipitate), (H & E).

A biopsy from an area on the right knee, including the edge of a necrotic ulcer, showed fibrinoid necrosis of small vessels in the ulcer bed. The adjacent dermis contained clusters of dilated capillaries, some containing eosinophilic deposits as seen in the other biopsies, but these channels were less distended and showed no tendency to nodular proliferation. There was a moderately heavy, mixed inflammatory reaction in the dermis and some fresh haemorrhage.

Two recent biopsies from areas of scarring on the upper arm showed an atrophic epidermis with dense dermal collagen lacking elastic fibres, at the periphery of which was a mild perivascular lymphocytic infiltrate with no evidence of any intravascular precipitate.

No amyloid was detected in any of the skin biopsies.

Direct immunofluorescent studies on all the above skin biopsies were either negative or non-specific.

DISCUSSION

Her severe chronic skin condition is undoubtedly related to the cryoglobulin which, although present at a relatively low concentration, is completely precipitated at 4°C and 50% precipitated at room temperature. The cryoglobulin has been classified as a monoclonal IgG kappa (type I) (Brouet *et al.*, 1974). Despite thorough investigation, no underlying disease could be found to explain the presence of the cryoglobulin. This, together with a history extending over 25 years, led us to regard this patient as having 'essential cryoglobulinaemia' (Volpe *et al.*, 1956). Ulceration and purpura were the predominant features in our patient. The ulcerated lesions were of long duration and some areas resembled pyoderma gangrenosum. The vasculitic, reticulate erythematous lesions on the face and limbs had a shorter history, but recurred frequently during the period of observation. Our patient had severe necrotising angiitis and also developed multiple benign angiomatous lesions on the face, earlobes and neck. There has been one previous case report of atypical haemangiomatosis of the skin associated with a paraproteinaemia by Oehlschlaegel, Stollman & Schropl (1968). Angiomatous proliferation, in association with cryoglobulinaemia, has also been noted by Ellis (1964) and Baughman & Summer (1966). The lesions seen in our patient showed an extravagant vascular proliferation with the appearance of individual collagen fibre dissection, which is said to be characteristic of a well-differentiated angiosarcoma (Rosai *et al.*, 1976). They are, however, completely benign.

Masson (1923) was the first to describe a bizarre endothelial proliferation associated with organizing thrombi, which he called 'vegetant intravascular haemangioendothelioma'. In 1932, Henschen interpreted this endothelial proliferation as a reactive process rather than a neoplasm. The process of thrombus organization has also been called 'intravascular angiomatosis' (Salyer & Salyer, 1975). The key microscopic feature is the presence of a capillary growth of hyperplastic endothelial cells supported by delicate fibrous stalks, entirely confined within the vascular lumen (Rosai & Akerman, 1974; Kuo, Sayers & Rosai, 1976; Clearkin & Enzinger, 1976). The lesions seen in cryoglobulinaemia are very similar and occur as a result of vessel blockage by cryoprecipitate with subsequent organization.

The factors thought to be important in the pathogenesis of her angiitis include: the presence of a cold precipitable protein, stasis and an increased plasma viscosity. Her serum viscosities were normal and gave effectively the same values in November 1981 and July 1982. The plasma viscosities, however, showed a marked increase with increased temperature dependence and were always significantly higher than the normal increment of plasma over serum would suggest. Two-dimensional immunoelectrophoresis showed a large increase in fibrinogen and fibronectin between these two dates. This disproportionate fibrinogen effect suggests that as the cryoglobulin matrix forms on cooling, it is entrapping the fibrinogen and fibronectin molecules, thus strengthening the matrix giving higher resistance to shear (or flow) and thus higher viscosity.

With regard to treatment, no specific therapy has been found to be of value in cryoglobulinaemia. Management is symptomatic and measures should obviously be taken to avoid the cold. Plasmapheresis may be beneficial in the acute situation (James & Kingston, 1979). Our patient has remained reasonably well, but if she should relapse this would be the treatment of choice.

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Persistent vegetating and keratotic lesions in patients with pemphigus vulgaris during immunosuppressive therapy

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SUMMARY

We describe three patients with pemphigus vulgaris, who during treatment with low doses of immunosuppressive drugs developed persistent keratotic or vegetating skin lesions. Direct immunofluorescence (IF) examination of these lesions showed the typical findings of pemphigus.

Oppenheim & Cohen (1942) described three untreated patients with pemphigus vulgaris, two of whom showed 'crusts of various forms'. Duperat (1959) mentioned the development of localized crusting lesions during the course of pemphigus in patients who were receiving corticosteroid treatment, and the occurrence of vegetating eruptions during the treatment of pemphigus vulgaris was also mentioned by Sneddon (1979). We now describe three further cases, with immunological investigations.

CASE REPORTS

Case 1. Male, aged 60 years. This patient with classical pemphigus vulgaris was treated with prednisone for 8 years and the dose was increased to 35 mg/day during an exacerbation of the disease. He was admitted with the diagnosis of prednisone-induced psychosis, at which time he was taking 30 mg of prednisone daily. He had at that time extensive keratotic and vegetating lesions around the mouth and chin. No bullae were present.

Case 2. Female, aged 75 years. This patient was being treated for pemphigus vulgaris for 2 years and was taking prednisone and azathioprine. Four months after discontinuing prednisone a relapse occurred. Biopsies were taken when the patient was receiving 15 mg of prednisone on

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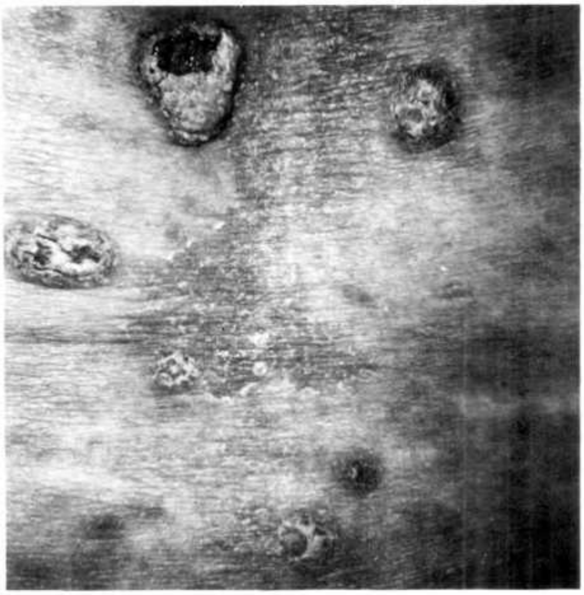

FIGURE 1. Keratotic lesions in the lumbo-sacral region.

alternate days and 100 mg of azathioprine daily. Keratotic lesions were present in the lumbo-sacral region (Fig. 1).

Case 3. Male, aged 47 years. He had been known to have pemphigus vulgaris for at least 13 years. Exacerbations occurred 6 and 9 years after the onset of the disease. His pemphigus was

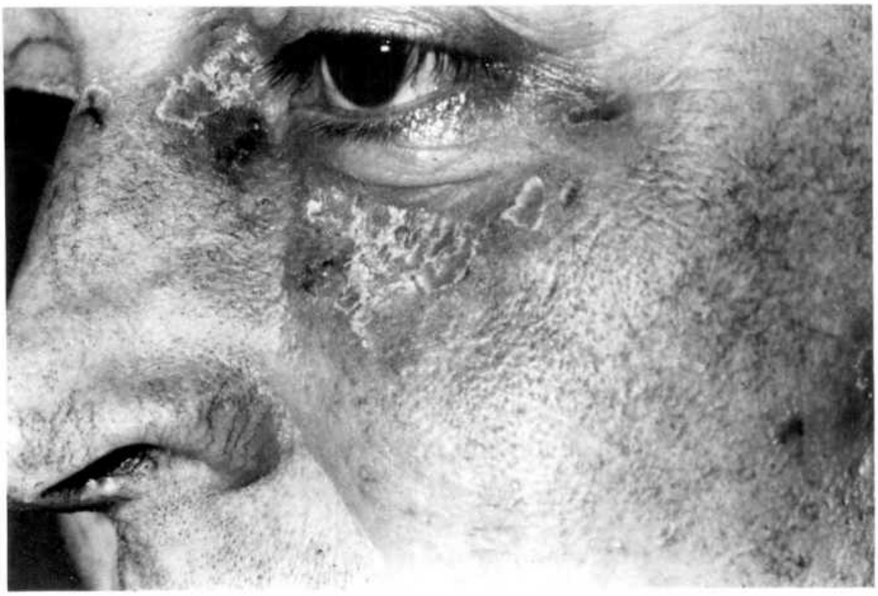

FIGURE 2. Keratotic lesions below the left eye.

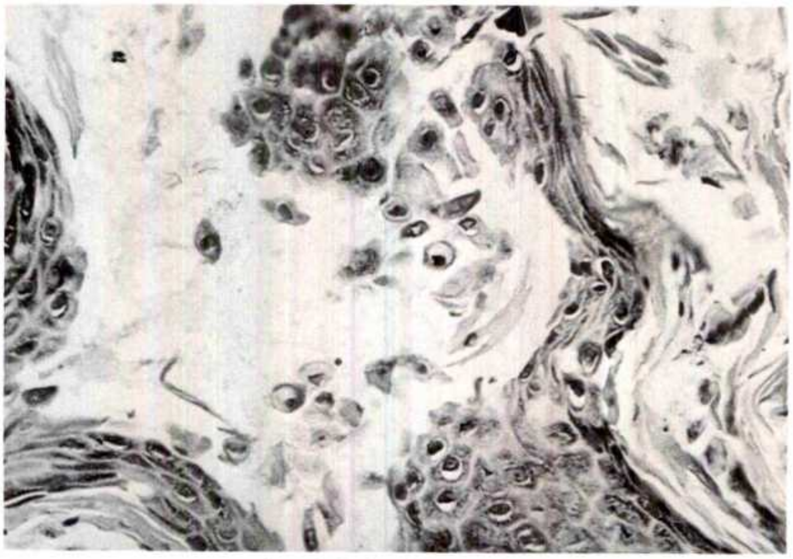

FIGURE 3. Acantholytic cells in the keratotic layer.

well controlled with 3½ tablets of Kenacort® on alternate days and a daily dose of 100 mg of azathioprine. Keratotic lesions were found under both eyes (Fig. 2).

Biopsies from the skin lesions were taken. Some were processed for routine histology and others were snap-frozen in liquid nitrogen for direct immunofluorescence (IF) examination. Commercially available, light-chain absorbed FITC-labelled antihuman IgG, IgM, IgA, IgE and C3 were used. Slides were read with a Leitz Orthoplan fluorescence microscope with blue narrow band excitation and epi-illumination.

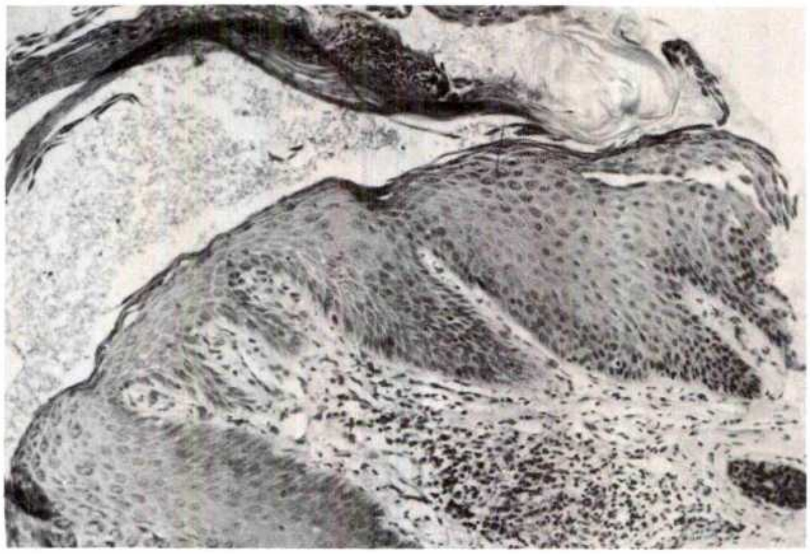

FIGURE 4. Downward proliferation of the rete ridges.

TABLE 1. Immunofluorescence and histopathological findings

	DIF (intercellular staining)								
	Lesional skin		Normal skin		IIF	Histopathology			
Patient no.	IgG	C3	IgG	C3	Serum	Acantholysis	Acanthosis	Eosinophils	Melanophages
1	+	±	−	−	−	+ +	+ +	±	+
2	+	±	+	−	n.d.	+	+	−	+
3	+	−	±	−	n.d.	+	+	±	+

n.d.: not done.
+ : positive fluorescence.
± : weak fluorescence.
− : no flourescence.

+ + : strong.
+ present.
± : a few.
− : not present.

Acantholysis was found in all cases and was visible in the upper epidermis (Fig. 3). A downward proliferation of the rete ridges was also present (Fig. 4). Eosinophils were sparsely present in two of the cases and were absent in the third. A considerable number of melanophages were found in the superficial dermis. Direct IF examination of the biopsies showed intercellular IgG deposits in all cases and sparse intercellular C3 in two cases. In the normal skin of the forearm only intercellular IgG deposits were found in two of the cases. The results are summarized in Table 1.

DISCUSSION

Although crusting, keratotic or vegetating lesions have been previously described in the course of pemphigus vulgaris (Oppenheim & Cohen, 1942; Duperat, 1959; Sneddon, 1979) detailed information has been lacking on these cases. In several papers describing large numbers of patients with pemphigus vulgaris treated with immunosuppressive drugs, this phenomenon was not mentioned (Lever & Schaumburg-Lever, 1977; Razzaque Ahmed, 1980; Judd & Lever, 1979; Fellner, Katz & McCabe, 1979; Burton & Greaves, 1974). The recently described acanthosis nigricans developing in resolving pemphigus vulgaris (Coverton & Armstrong, 1982) is different from the lesions we observed. In our three patients, vegetating or keratotic lesions were seen in locations where they are not normally found in pemphigus vulgaris (Degos, 1953). Histopathological examination of the lesions in our patients showed acantholysis, a feature of pemphigus. Hyperplasia of the epidermis, an important feature of pemphigus vegetans, was also present, but this finding can also occur in pemphigus vulgaris (Lever & Schaumburg-Lever, 1975). Eosinophils, which tend to be numerous in active pemphigus vegetans, were almost completely absent in our cases. The presence of a considerable number of melanophages in the superficial dermis was a conspicuous feature of this study.

IgG deposits in the intercellular spaces of the epidermis were found in all three cases. Intercellular C3 deposits were found, although to a lesser extent, in two of the patients and these two patients had recently experienced an exacerbation of the disease. The disappearance of intercellular C3 deposits during successful treatment in a case of pyodermite vegetante has also been observed (Neumann & Faber, 1980). The clinical and histopathological findings in these patients with pemphigus vulgaris are suggestive of pemphigus vegetans and provide further support for the concept of a spectrum of pemphigus.

Although different percentages for the C3 deposits in perilesional skin in cases of pemphigus have been reported by various authors (Van Joost, Cormane & Pondman, 1973; Bystryn, 1981; Kumar & Beutner,1981) one may speculate that the absence or presence of C3 deposits in the type of lesions described in this paper is an indication of the activity of the disease. The question arises as to whether the presence of keratotic or vegetating lesions in pemphigus vulgaris patients is only a harmless sequel or is a sign of active disease. The determination of the presence of C3 deposits in these lesions may help to answer this question. Furthermore, it may have implications in the determination of the most suitable treatment regime for this disorder.

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Comment

GENETIC ENGINEERING AND GENE ANALYSIS

The term genetic engineering refers to the spectacular recent advances in DNA biochemistry which have allowed, in almost miraculous fashion, human, animal and plant genes to be analysed, 'finger-printed,' sequenced, transferred, cultured and otherwise manipulated (Seltnor & Hollander, 1979). The potential implications for medicine, agriculture and society in general are far-reaching and perhaps ultimately as important, frightening and revolutionary as atomic fission has been upon the future of mankind in general. The immediate tangible results have been in classical human genetics and protein synthesis. Genes have been inserted and amplified in bacteria and the isolated genetic products have either been used for sophisticated genetic analysis or to instruct bacterial cells (Crea *et al.*, 1978) to make desirable products such as insulin (Keene, Glynne & Picking, 1980) or human interferon (Goeddel *et al.*, 1981). Future expansion of such gene mapping technology should allow the detailed understanding of gene regulation and switching. If this can be achieved then the control and manipulation of relatively simple single gene defects such as the haemoglobinopathies, inherited defects of collagen and many others is virtually certain. Equally important possibilities include a complete understanding of the genes controlling the HLA protein system and the changes produced in certain animal cell lines by certain viral or virus-like genes which are associated with the uncontrolled cell proliferation or behaviour which we recognize and fear as carcinogenesis. Unhampered organ transplantation, the control of some cancerous processes, the replacement or regulation of human structural gene products and a much more sophisticated understanding of all sorts of regulating and switching capabilities of various cellular processes such as lymphocyte T- and B-cell activation could revolutionize the practice of medicine as we understand it today.

What is available now?

At present the main successes of this approach have been upon gene structure and regulation allowing accurate genetic counselling and highly sophisticated prenatal diagnosis in the haemoglobinopathies, especially sickle cell disease and α and β thalassaemia (Weatherall, 1979). Recognizable genetic pathology here has included partial or complete gene deletions (Maniatis *et al.*, 1980), translational abnormalities of mRNA producing chain lengthening or shortening abnormalities (Proudfoot & Brownlee, 1976) (from faulty stop codons) and most recently intron splicing abnormalities (Busslinger, Moschonas & Flavell, 1981). Although correction of these abnormalities is not yet feasible there is a real possibility that it may become so in future. Similarly, the biosynthesis of human enzymes, hormones, vaccines and neurotransmitters is in its infancy so far, but rapid progress in this field can be expected in the near future.

Technical aspects

Watson & Crick's (1953) discovery that DNA consists of a series of nucleotide bases contained in two intertwined helical strands, each the exact complement of the other, is the foundation upon which the whole of modern DNA technology depends. From this came the discoveries that nuclear DNA is converted into a series of intermediates ending as the final protein product and that each amino acid in the protein chain is coded by a particular base triplet sequence in the

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DNA (genetic code) (Crick *et al.*, 1961). Specialized messenger, transfer and ribosomal RNA molecules then organize protein production. These discoveries were brilliant in their conception and execution and explained rationally the previously metaphysical concepts of genes and heredity. Then fundamental research into transferable and transmittable bacterial genetic elements such as plasmids and phages led to the realization that they could be modified to transmit or carry virtually any animal or plant gene. This, together with the discovery that certain bacterial restriction enzymes are capable of cutting DNA sequences at specific points, has allowed the fingerprinting and analysis of animal and plant DNA. It has also allowed the biological tailoring of animal, plant and carrier DNA so that foreign genes can now be inserted into suitable carriers or vectors. These can then be used to 'infect' bacteria with any desired gene fragment, and the latter can be multiplied by using conventional bacterial cultural methods. Any desired gene can then be selected and amplified when required, provided that there is some means of identifying it. This process is called gene cloning.

Analytical procedures

(1) *DNA isolation.* DNA is easily made for analytical purposes from citrate dextrose anticoagulated blood or cultured skin fibroblasts followed by proteinase K/SDS digestion, phenol extraction and alcohol precipitation (Little, 1981).

(2) *Restriction endonuclease analysis.* This relies upon the capacity of certain bacterial enzymes to cut foreign (non-bacterial) DNA at specific sequences. Several hundred enzymes are now known (Roberts, 1980) and they vary from those which make few cuts, e.g. Bam HI or Eco RI, cutting at G/GATCC and G/AATTC respectively, to Taq and Mbo I which produce many more fragments and cut for example at T/CGA. Particular restriction enzymes are selected for their specificity and are generally utilized in one of two ways: either to generate gene fragments for DNA analysis or to engineer fragments for insertion and cloning (multiplication) in bacterial carriers (vectors).

Southern blotting

Named after its creator (Southern, 1975), this procedure allows the transfer of electrophoretically separated DNA fragments in the double stranded mode into a single stranded form covalently attached to nitrocellulose membranes after alkali denaturation and neutral salt fixation at pH 7·5. The technique is particularly suitable for the demonstration of specific human gene fragments which are then 'lit-up' with complementary radioactively-labelled probes. The method has been spectacularly successful in the understanding of the human haemoglobinopathies, especially α (Orkin *et al.*, 1979) and β thalassaemia (Flavell *et al.*, 1979) where it has convincingly shown specific gene deletions. The method has recently been applied to human growth hormone deficiency, where a homozygous 7·5 kb deletion has been shown in isolated growth hormone deficiency type A (Phillips *et al.*, 1981). This approach should also be fruitful in rare genetic diseases like calcitonin deficiency and various inherited defects of collagen.

Messenger RNA (mRNA) analysis

Messenger RNA (mRNA) mediates the conversion of the genetic information contained within nuclear DNA into its final extracellular (sometimes intracellular) protein form. The message, like its DNA template, is a long series of nucleotide bases and is the complement of the gene

DNA (Spiegelman, 1964) except that uridine takes the place of thymidine. The message is a shortened (edited) version of the gene which contains both coding and non-coding sequences. The coding sequences (exons) are actually incorporated into the final protein product, whereas the non-coding intervening sequences (introns) alternate with the coding sequences or exons (Crick, 1979). The process whereby the coding sequences are read from the gene and joined together to form the final mature message is called splicing. The conversion of DNA to message is called transcription and the conversion of the spliced message into protein with the active and other specialized transfer and ribosomal RNAs is called translation. Genetic abnormalities in any of these steps can cause either little or no protein production or the formation of normal or abnormal quantities of a mutant protein.

Most of these stages can now be studied in various pathological human and animal situations. For example, in the haemoglobin thalassaemias, abnormalities of message production (Wood *et al.*, 1978), premature or late-stop codons which shorten or lengthen the mutant protein chains are well known. RNA can also be sized and sequenced, the former by a blotting technique analogous to Southern blotting (humorously called Northern blotting). RNA sequencing is carried out by radioactively labelling one or the other end (3′ or 5′ end) of the sequence and then digesting with base-specific endonucleases such as ribonuclease T, U_2 or I. It can also be sequenced by chemical modification of one of the four bases and splitting at the modified site (Peattie, 1979). Another alternative is to make a cDNA copy of the RNA sequence with reverse transcriptase and then sequence the DNA copy with standard DNA sequencing techniques.

DNA sequencing

Maxam & Gilbert (1980) and Sanger & Coulson (1977) independently conceived and executed methods of determining the exact order of bases contained in genes of [^{32}P]-labelled DNA. The Maxam & Gilbert method uses polynucleotide kinase to insert ^{32}P at the 5′ end of the DNA sequence. The assay conditions are so arranged that the radioactively-labelled DNA will be split at every adenine, guanine, cytosine or thymine with individual assays specific for each base. If, for example, adenine is the splitting point then a series of radioactively-labelled bands with lengths depending upon the position of the split and labelled nucleotide will be obtained. The sequence can then be read off in a series of four tracks in order of molecular size. Sanger's method uses DNA polymerase I to copy single stranded DNA after the formation of a restriction enzyme-generated priming fragment. The conditions are so arranged that the copying will prematurely stop at either A, C, G or T by using analogues of each of these bases which can be radioactively incorporated in the correct position, but which fail to link with the next base in the sequence. This produces a series of radiolabelled DNA copies ending at random at each A, C, G, T in the sequence. These [^{32}P]-labelled bases are then separated by size on a polyacrylamide gel and the gene sequence directly read from the pattern generated. Using this method Sanger sequenced the entire 5000 base pair sequence of the bacterial plasmid ϕ174. Similar studies are now routine in the haemoglobinopathies (Spritz *et al.*, 1981) and will soon become so with the other protein defects, such as inherited abnormalities of collagen.

Cloning

This is the process by which foreign DNA (human, animal, or plant genes) can be introduced into suitable vectors and then incorporated into and reproduced by bacterial cells—usually *E. coli*. Standard bacterial culture will then produce multiple copies of these chimaeric genes. Two types of clone are available: genomic in which the whole DNA is sheared or restriction enzyme digested, and cDNA in which mRNA is copied with reverse transcriptase into DNA. The latter contains only coding sequences (exons) as all the introns have been spliced out in the production of the message. There are advantages and disadvantages to each. Generally genomic clones are easier to make but have at least ten times as many fragments to be screened. Also the screening procedure is less reliable with genomic probes, as false-positive cross hybridization with poly dT sequences and ribosomal RNA is a problem. cDNA clones are useful in determining the internal gene sequence organization and also are important for expressing gene eukaryotic (animal or plant) products in bacteria (prokaryotes), as bacterial genes lack introns and bacteria cells are not able to translate intron-containing genes (Williams, 1981).

Making a clone generally involves three major steps:

(a) Constructing a compound (recombinant) animal/vector DNA.
(b) Introducing the hybrid DNA into host bacterium.
(c) Selecting only those cells containing the relevant gene for subculture and amplification.

The vector (cloned gene carrier) must replicate easily within the host bacteria; plasmids, phages or cosmids are generally used for this task. Plasmids are extrachromosomal transmissible circular DNA particles, phages are bacterial viruses and cosmids are plasmid-like particles possessing phage-like cohesive ends which allow them to be packaged into suitable phage carriers. For transmission into bacteria, foreign DNA is best packaged within one of these vectors although both prokaryotic and eukaryotic cells can spontaneously absorb small quantities of foreign DNA. Those vectors which have incorporated the foreign (cloned) DNA must then be selected by their ability to confer upon the infected bacteria recognizable properties which can be used to select for the relevant gene sequence.

For genomic clones, human chromosomal DNA is sheared either with restriction enzymes or by high speed centrifugation (Dahl, Flavell & Grosveld, 1981). The randomly sized fragments can then be joined to the carrier DNA vector with a variety of techniques. These include the spontaneous production of complementary sticky ends in carrier and target DNA and the synthesizing linkers which join the two DNA species. In the case of the enzyme-generated sticky ends, the plasmid/fragment hybrid is joined by DNA ligase and in the linker method, enzymes such as terminal transferase synthesize the extensions, DNA polymerase forms the gaps and DNA ligase seals the ends (Dahl *et al.*, 1981).

Usually the plasmid or phage vectors are so constructed that particular restriction enzymes can both excise the inserted sequence (from say human DNA) and simultaneously produce within the vector a gap sufficiently large to insert the sequence of interest. By selecting carriers (plasmids or phages) in which the removal of DNA segment activates or inactivates a gene for antibiotic resistance or susceptibility (often to ampicillin or tetracycline) then vectors into which a desired sequence has been inserted can be recognized. Thus for example, the plasmid pBR322 contains genes for ampicillin and tetracycline resistance which can be transferred to *E. coli* which is normally sensitive to these antibiotics. If a gene insert is then linked to the plasmid DNA in such a way that the tetracycline resistance gene is inactivated, *E. coli* with plasmids containing this gene insert can easily be selected. Bacteria without the plasmid will be tetracycline and ampicillin sensitive. Those with plasmids containing the required gene fragment will be tetracycline sensitive. Bacteria containing plasmids which have failed to insert the gene will be tetracycline and ampicillin resistant (Struyer, 1981). Lambda phage vectors are selected by their capacity to be packaged and reproduced in bacteria only when a suitable DNA insert has been added to them. Phages with cloned DNA inserts are then selected by their ability to lyse bacteria. A variety of mutants are now available and inserts as large as 40,000 bases (40 Kb) long can be carried by them without destroying the ability to multiply and destroy bacteria. The infected phages are then identified by the lysed areas on bacterial culture plates which are then screened for particular genes with [^{32}P]-labelled DNA or RNA probes.

Applications

The potential general applications for this type of technology are extremely varied and include the following possibilities.

(1) Gene mapping, sequencing, and the regulation of control and transcription.
(2) As a direct result of (1), prenatal diagnosis and reliable genetic counselling.
(3) The possible treatment, replacement or regulation of faulty genes, depending upon the information obtained from (1) or (2).
(4) The synthesis of rare human proteins or peptides, e.g. insulin, somatostatin, calcitonin or neuropeptide transmitters.
(5) The selection of desirable genes and their insertion into genomes from which they have previously been absent, e.g. the insertion of nitrogen fixation genes into non-leguminous plants.

The haemoglobinopathies are an excellent example of how these techniques illuminate and clarify our molecular understanding of single gene disorders. Southern blotting of the haemoglobin gene cluster has successfully identified complete and partial gene deletions of the α, β, γ genes (Little, 1981). It has also shown that random extra gene sequence differences when close enough to the gene are sufficiently common to allow the diagnosis of genetic diseases which are inherited along with them. Allele specific polymorphisms are those in which a majority of affected individuals show the sequence change; a good example is sickle cell disease which is associated with a Hpa I polymorphism in 87% of American Negroes of African extraction (Kan & Dozy, 1978). Linked polymorphisms are similar sequence differences occurring in a minority of individuals with a disease, but breeding true within those families. This allows useful predictions of disease within but not outside the family group and has been successful in studies of sickle cell disease as well as β thalassaemia (Little, 1981).

Polymorphisms could also be used to identify homozygotes and heterozygote carriers of diseases in which the gene and protein defect are currently unknown. All that is necessary is that the chromosomal mapping position of the disease should be known. Provided this is so then individual chromosomal clones can be constructed and used in Southern blotting experiments with the diseased or unknown human DNA. If polymorphism associated with the gene can be consistently identified then accurate genetic counselling and confident intra-uterine diagnosis are possible. This approach would be particularly important in relatively common diseases like cystic fibrosis and Duchenne muscular dystrophy but is applicable in principle to any inherited disease.

Possible dermatological applications

Listed below are the types of dermatological diseases to which these techniques may be applicable. Generally these fall into several groups—the relatively simple single gene disorders and the more complicated polygenic defects and also cancerous processes of various kinds.

Single gene disorders. Any disease in which an altered protein chain is known or considered likely is a potential candidate for these types of study. Provided probes are or will become available the actual genetic abnormality is potentially identifiable and even theoretically treatable. Obvious examples in the skin are the predominating epidermal and dermal proteins including keratin, collagen, elastin and laminin. Diseases which could be due to genetic abnormalities of these substances are listed below.

Keratin proteins: the ichthyoses, various inherited abnormalities of hair keratins, nail defects (including pachyonychia congenita, inherited koilonychia, and the nail patella syndrome) and multiple ectodermal dysplasia.

Collagen proteins: Ehlers-Danlos syndrome (collagens I and III), neurofibromatosis, tuberous sclerosis, epidermolysis bullosa, progeria and Werner's syndrome.

Elastin: pseudoxanthoma elasticum, cutis laxa.

Laminin: epidermolysis bullosa and nail patella syndrome.

Abnormalities of regulation and control. Here the mechanisms of causation are obscure but the diseases are characterized by an increase or decrease in the production of particular proteins.

Keratin: psoriasis, pityriasis rubra pilaris, male pattern alopecia, alopecia totalis.

Collagen: local and systemic scleroderma, keloid formation and scarring, steroid atrophy and premature and normal skin ageing.

Cutaneous cancers. Squamous cell carcinoma, basal cell carcinoma, Bowen's disease, malignant melanoma, cutaneous lymphomas.

Although environmental exposure to numerous organic and inorganic carcinogens can undoubtedly cause cancer there is good evidence for genetic predisposition to at least some cancerous processes. An excellent dermatological example is the so-called familial atypical multiple mole (FAMM) syndrome in which predisposition to malignant melanomas and probably other malignant conditions is inherited as a variable dominant (Lynch *et al.*, 1980). DNA recombinant technology has also encouraged exciting recent advances in the understanding of the link between certain animal viruses and malignant tumour promotion. These so-called oncogenes are transmitted in viral genomes making them capable of malignantly transforming previously normal cells (Bishop, 1982). The oncogenes act by inducing intracellular phosphorylases which then alter cell membrane properties so as to make the transformed cells non-contact-inhibited and invasive. It is entirely possible that other (non-oncogene) viruses may also integrate into normal healthy cellular DNA and widely differing carcinogens may produce all sorts of subtle alterations of cellular properties. Already a specific and crucial difference has been discovered by two American groups (Tabin *et al.*, 1982; Reddy *et al.*, 1982) in oncogenes coding for the so-called p21 protein in human bladder epithelial cells from normal and carcinomatous tissue. The carcinoma-associated gene differs from normal by only one amino acid, with glycine in position 12 becoming mutated to valine. So far the function of p21 protein is completely unknown and both small doses of the mutated protein or large doses of the normal protein can transform mouse cells to become non-contact-inhibited. The precise link between the mutated protein and the actual production of carcinomatous properties is of course obscure but it is certain that other human malignancies including those affecting the skin will prove to have similar mechanisms.

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Society Proceedings

Proceedings of the 226th Meeting of the Netherlands Society for Dermatology and Venereology, Amsterdam, 29 January 1983, University of Amsterdam

PRESIDENT: PROFESSOR E.VAN DIJK
HOST: PROFESSOR R.H.CORMANE

Theme: Eye and Skin (joint meeting with the Netherlands Ophthalmic Research Institute)

SUMMARIES OF PAPERS

The eye and the skin: introduction and study of necrobiotic xanthogranuloma with paraproteinemia

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The skin and eye are complex ectodermal organs that are associated with multiple nervous, vascular, connective tissue and glandular structures. These multiple structures of the skin may now be studied as isolated structures and their function and importance related to the eye also. Genetic, immunological, metabolic, degenerative, and neoplastic changes in eye and skin may indicate similar or diverse changes in systemic disease. An example of the relation is necrobiotic xanthogranuloma. This disease may begin as an apparent xanthelasma or other type of xanthoma. Table I gives a differential diagnosis of normolipaemic xanthomatoses. The eye and skin lesions were infiltrative, nodular, inflammatory, progressive, ulcerative and scarring xanthomatous lesions that involved periorbital tissue in all of the first eight patients reported. The eye may show keratitis, conjunctivitis, uveitis, iritis, proptosis, and blindness. The disease may be uni- or bi-ocular. The lesions on the skin usually have more inflammation, and develop more atrophy and irregularity with areas of xanthoma, than the lesions about the eye. Two patients had multiple myeloma, one a lymphoma on bone marrow examination, and two had marrow proliferation of atypical plasma cells. Subcutaneous nodules and distinctive inflammatory and xanthomatous plaques with atrophy and ulceration measuring up to 10 cm occurred over the trunk. Skin biopsy showed a combination of granulomatous and necrobiotic masses appearing as nodules or wide bands replacing much of the dermis and involving the subcutis. Giant cells of the foreign body and Touton type are present within the granulomas and also surrounding the areas of necrobiosis. Polarization failed to reveal foreign material, but cholesterol crystals were observed in some cases. Granulomatous invasion of muscular vessel walls with thrombosis was also observed. The presence of lymphoid nodules was striking in several cases. Patients reported with combinations of xanthoma and myeloma, xanthoma and leukaemia, xanthoma and monoclonal cryoglobulinaemia have this histopathology and presumably represent a common syndrome. Rare complement deficiencies and hyperlipidaemia have also been reported as part of this complex.

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TABLE I. Normolipaemic xanthelasma

Xanthelasma
Hyperbetalipoproteinaemia
Hyperapolipoproteinaemia
Cerebrotendinous xanthomatosis (cholestanol)
Sitosterolaemia
Histiocytic diseases
Leprosy
Reticulohistiocytoma cutis
Xanthoma disseminatum
Necrobiotic xanthogranuloma

Combining these manifestations in necrobiotic xanthogranuloma with paraproteinaemia is justified by current knowledge.

Evaluation of hairbulb incubation test and tyrosinase assay in the classification of albinism

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Hairbulb tests and tyrosinase assays were performed in eighty-eight albinos, fifty-nine obligate heterozygotes and fifty-four normal controls. Our findings suggest that the classification of albinism is more complicated than previously assumed. An improved classification system is important for genetic counselling as there is evidence for the existence of two different autosomal non-allelic genes for oculo-cutaneous albinism. Our results indicate that the hairbulb test alone is insufficient for classifying albinos, i.e. detecting two genotypes. The hairbulb test, tyrosinase activity, hair colour and the ability of the skin to tan do not run parallel in all cases.

We tried to classify the albinos phenotypically and biochemically, the latter into five groups: four according to the hairbulb test, a fifth group consisted of X-linked ocular albinism (XOA). Tyrosinase (T) assays demonstrate that the values range from zero to low in our TN (N = negative) and T ± groups; from zero to high in our TP+ (P = positive), TP and XOA groups. The latter values overlap with those of the controls and the heterozygotes. Because of this overlap we found that it is not possible to detect heterozygotes for tyrosinase negative oculo-cutaneous albinism (TNOCA). Comparing our data with those for the U.S.A. and the U.K. we found a difference in frequency of TNOCA in the Netherlands, i.e. five times less than expected.

Ultrastructure of the skin of human albinos

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As part of a combined genetic, electrophysiological, clinical and biochemical study of albinism, we have investigated the ultrastructure of the epidermis of forty-six albinos. On account of the amount of pigmented granules in the keratinocytes, presence of distinct maturation stages of melanosomes, occurrence of abnormal melanosomes in the melanocytes and keratinocytes and the packing of melanosomes in these keratinocytes, we can distinguish three groups:

Non-pigmented. The keratinocytes of these patients contain only premelanosomes stage I and II and completely lack pigmented granules.

Variably pigmented. The keratinocytes of these patients contain stage IV melanosomes in variable number. Premelanosomes stage II and III can be present.

Pigmented with macromelanosomes. The epidermis of these patients is characterized by the occurrence of macromelanosomes.

In addition, the keratinocytes contain aggregated and non-aggregated melanosomes.

Comparison of the ultrastructural observation with clinical diagnosis according to Witkop, Quevedo & Fitzpatrick (1978) revealed that non-pigmented epidermis is present in patients in which the diagnosis of tyrosinase-negative oculo-cutaneous albinism, tyrosinase-positive oculo-cutaneous albinism or yellow mutant oculo-cutaneous albinism was made. Because pigmented moles are present in both tyrosinase-negative OCA and tyrosinase-positive OCA albinos this criterion cannot be used in discriminating between tyrosinase-negative OCA and tyrosinase-positive OCA.

We found a better concordance between the clinical phenotype and the skin ultrastructure, than between the phenotype and the hair bulb test.

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An electrophysiological correlate of human albinism

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The consequences of the albino mutation are widespread, producing not only hypopigmentation of the hair, skin and eye but also sensory system anomalies particularly of the visual system. Characteristic features of albinism include reduced visual acuity, photophobia, nystagmus and visual pathway misrouting. The misrouting is in the form of temporal retinal fibres which abnormally decussate along the visual pathway. The resultant abnormal retino-geniculo-cortical projections can be observed in the visual evoked potential (VEP). The basic electrophysiological feature in albinos is VEP asymmetry across the occiput following monocular stimulation. Under optimal test conditions we have found VEP asymmetry in all albinos with measurable responses. There were no false positives in non-albino controls including heterozygote family members and in those controls with comparable ophthalmic symptoms. Our test sample to date includes over 100 albinos. The 100% detection rate of VEP asymmetry in albinos and the absence of false positives in normal controls indicates that VEP asymmetry is a decisive clinical measure for the diagnosis of albinism and for differential diagnosis. The protocol for electrophysiological diagnosis and the results of its application in several atypical patients are presented.

SUMMARIES OF POSTERS

Neurofibromatosis and hypertelorism

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Of thirty-four patients with neurofibromatosis (Von Recklinghausen) who were seen in the Departments

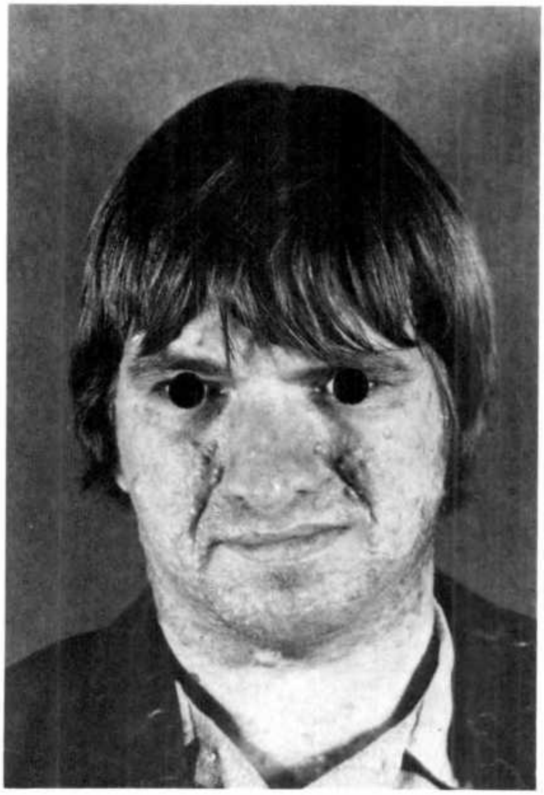

FIGURE 1. Hypertelorism in neurofibromatosis.

of Dermatology and Neurology of the Academisch Medisch Centrum, University of Amsterdam, and the Interuniversitair Ophthalmologisch Instituut, hypertelorism was observed in eight. The diagnosis was made by measuring the intercanthal distance and this was related to the biparietal distance of the skull. The data were compared with those of members of their respective families. X-ray examination of the orbit was difficult to interpret.

Hypertelorism, apart from an isolated phenomenon, is seen in many syndromes which can be subdivided into three main groups (De Meyer, 1967):

(1) Median cleft face syndrome (facial dysostosis);
(2) Cranium bifidum (occultum) syndrome;
(3) Syndromes in which there is an association with other mesodermal disturbances.

Hypertelorism, which is congenital, has not been described in association with neurofibromatosis before (Fig. 1).

In our group of patients we ruled out facial dysostosis and cranium bifidum occultum (meningocele, meningo-encephalocele, nasal lipoma). Megalocephalia due to hydrocephalus did not occur.

Apart from the highly prevalent skeletal abnormalities like kyphoscoliosis (Hunt & Pugh, 1961) and hypo- and hyperplasia of one part of the body in neurofibromatosis patients, some rather specific radiological changes of the skull have been described (Binet *et al.*, 1969; Tänzer, 1966).

(1) Excavation of the sella tursica;
(2) Enlarged middle cranial fossa;
(3) Deformity of the orbit.

Most bones of the base of skull and of the face are mesenchymal structures (supportive tissue) although derived from the neural crest. It is quite possible that the above mentioned deformities, including hypertelorism, are caused by a developmental disturbance of the neural crest.

The high prevalence of hypertelorism in our group of patients (24%) makes it likely that this easily recognizable clinical symptom is associated with neurofibromatosis. Its presence at birth ranks it in the category of early diagnostic criteria, which may assist with genetic counselling.

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Eye involvement in leprosy: a survey among patients in The Netherlands

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Leprosy affects the eye in two ways:

(a) Indirectly: through invasion of *M. leprae* into the fifth (N V) or seventh (N VII) cranial nerves, and immune response reactions in the nerve, causing lid weakness, lagophthalmos and possibly damage to the cornea by exposure keratitis, and corneal anaesthesia, which may worsen corneal damage, respectively.

(b) Directly: through invasion of *M. leprae* into the eye and immune response reactions, or via circulating immune complexes (ENL). Chronic iritis is said to be the most frequent cause of blindness in leprosy. LL- and BL-patients are especially prone to direct eye involvement. In 1979/80 a survey was done among 121 out-patients visiting the leprosy clinic of the Department of Dermatology, Amsterdam Medical Centre (former Binnengasthuis).

Among forty-three TT-classified patients one patient had madarosis (loss of eyebrows) due to a reactive midline patch on his forehead. None had eye lesions.

Among twenty-four BT-patients were eight cases of lid weakness and lagophthalmos due to N VII paresis and/or corneal anaesthesia due to N V lesions. A slight decrease of vision by corneal damage was found in three patients. In nine patients classified as BB one patient (age 48; known leprosy for 41 years) had several direct eye lesions and had probably been a BL-case before. No other BB-case showed eye involvement. Among twenty-five BL-classified patients six showed madarosis only; three had signs of direct eye involvement. Twelve out of twenty LL-patients showed some kind of eye involvement (mainly corneal opacities, iritis or iris atrophy), two had madarosis only and only six were without detectable eye lesions.

The BL- and LL-patients had a history of leprosy of more than 20 years and were over 40 years of age on average. Only seven eyes (< 4%) of five patients had a visual acuity of 0·2 or less, all due to chronic or acute iritis; four patients had longstanding LL of at least 28 years; one BL-patient had a history of leprosy for 55 years.

In conclusion, potentially blinding lesions were found only in longstanding LL- and BL-cases in this series. Lagophthalmos was the most frequent lesion in BT-patients.

Dermatology Session

SUMMARIES OF PAPERS

Primary management of cutaneous melanoma: the role of the dermatologist

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In 1981 a study was undertaken in the Amsterdam and Rotterdam area on the patient's and doctor's delay in treating cutaneous malignant melanoma. The study was performed under the auspices of the Amsterdam Melanoma Working Group. The first 150 melanomas entering the study are the subject of this report. A total of eighty-four consultations by dermatologists were recorded: twenty-one melanomas were seen in an academic dermatology department, whereas sixty-three melanomas were seen by non-academic dermatologists.

Primary management in the academic institution nearly always consisted of immediate referral to the surgical department of the hospital. Only in two cases was punch biopsy performed. In both instances the clinical suspicion of melanoma was very low.

Non-academic dermatologists frequently resorted to restricted diagnostic or therapeutic procedures (29 patients, 46%), including punch and incisional biopsy, electrocautery, currettage, etc. Some of these melanomas may have been of low clinical suspicion or so situated that primary excision would be mutilating or disfiguring. Nevertheless, there appears to be a striking difference in approach between academic and non-academic dermatologists.

It is emphasized that the initial diagnostic procedure in suspected melanoma should encompass the complete removal of the lesion (excisional biopsy). This will enable the pathologist to report accurately on the histological prognostic factors such as the Breslow-thickness. Moreover, there are indications that initial 'maltreatment' may jeopardize the patient's survival.

Pharmacological manipulation of the complement system

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Complement is a powerful effector system involved in the body's immunological defenses. In normal individuals it is present in a dormant state, its dormancy being a prerequisite for its capacity to defend the body. In many disorders, however, the complement system is found to be in an activated state and this activation has been shown to be associated with diseases of many organs, such as skin and eye diseases. A few examples of such skin diseases are SLE, pemphigus, pemphigoid, vasculitis, psoriasis, scleroderma and hereditary angioneurotic oedema. Some examples of eye diseases with evidence of complement involvement, or suspected complement involvement, are Morren's ulcers, pemphigus, cicatricial pemphigoid, scleritis and scleromalacia, corneal infiltrates and peripheral ulcers.

It is anticipated that complement inhibition will arrest the above mentioned disease processes. We are therefore concerned with the development of compounds that can inhibit complement *in vitro* and *in vivo*. In our laboratory diphenyldiamidines have been shown to inhibit complement at the C1 and C3 steps, phenothiazine and chlorophenothiazine sulphonates at the C3 step and certain phenylindandiones at the C5 step.

Inhibition of complement-mediated reactions in rabbits by certain complement inhibitors (2-hydroxystilbamidine, chloropromazine, chlorophenothiazine sulphonate and suramine) was tested using the Arthus reaction as a model. Chloropromazine inhibited erythema formation by 50% whereas other compounds tested inhibited it by 15–20%. Cellular infiltration at the site of the Arthus reaction was measured by means of a computerized area integrator. Suramin was found to inhibit cellular infiltration by about 65% whereas other compounds tested inhibited it by about 50%. Complement deposition, as tested by immunofluorescence techniques, was appreciably inhibited by 2-hydroxystilbamidine but less effectively so by other compounds. The most dramatic testimony of complement inhibition was furnished by electron microscopic studies on tissue injury at the Arthus reaction site. All four of the compounds gave protection against destruction of the endothelium and basal lamina at the Arthus reaction site.

These studies indicate that low molecular weight complement inhibitors can prevent complement-mediated tissue injury. The need for more specific, more potent and safer complement inhibitors is therefore emphasized.

Biopharmaceutical and pharmacokinetic aspects of some psoralen derivatives

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We administered various formulations of 8-methoxypsoralen (8-MOP) to patients. Serum concentrations were determined in order to find a formulation with fast and predictable absorption. Also the pharmacokinetic properties of 5-methoxypsoralen (5-MOP) and 4,5,8-trimethylpsoralen (TMP) were investigated. Capsules filled with 'micronized' 8-MOP, 'non-micronized' 8-MOP and 8-MOP as a solid dispersion in polyethyleneglycol 6000 were compared. None of the capsules complied with the requirement for fast and predictable absorption. High serum concentrations and fast absorption were observed after oral administration of 8-MOP in a supersaturated solution in water. Also fast absorption, but lower serum concentrations, were obtained with 8-MOP in an oil–water emulsion. Nausea was experienced more frequently after ingestion of the solution than with the capsules or the emulsion.

After rectal administration of 8-MOP in a microenema little nausea was experienced. Rectal absorption was very fast.

Saturable 'first pass' elimination existed after both oral and rectal administration.

Serum concentrations were measured after oral administration of 5-MOP. The pharmacokinetic profile of 5-MOP resembles that of 8-MOP, but as long as no dosage formulation of 5-MOP with fast and predictable absorption is available, 8-MOP should be preferred in PUVA therapy.

After oral administration of TMP, serum concentrations were very low or not detectable and much of the ingested dose was recovered unchanged in the faeces. Because of its poor absorption the effectiveness of TMP is doubtful.

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SUMMARIES OF SCIENTIFIC POSTERS

Thalidomide

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Thalidomide is, since the disaster of 1961, a controversial drug. In that year it was shown that thalidomide was responsible for a large number of fetal abnormalities which had occurred in previous years.

Since then thalidomide has been almost exclusively used for the treatment and prevention of erythema nodosum leprosum, a condition that occurs in multi-bacillary leprosy patients. Untreated, this condition may have serious consequences for the patient and after repeated attacks this may lead to severe disability due to nerve damage and blindness.

By 1980 it had become clear that thalidomide had a favourable effect on actinic prurigo and discoid lupus erythematosus. Since then many claims to the effectiveness of thalidomide in a wide variety of diseases have been recorded (Barnhill & McDougall, 1982; Hasper & Klokke, 1982; Naafs *et al.*, 1982).

In our department over the past 3 years we have used thalidomide to treat, under careful follow-up, thirty patients suffering from different forms of lupus erythematosus, vascultis, prurigo nodularis and other diseases. The poster summarizes the claimed therapeutic effects of thalidomide, its possible mode of action and our own results. Side-effects, e.g. dizziness, dry mouth, constipation, neuropathy etc. were seen in 60% of the patients. Since the side-effects seem to be dose-dependent we advocate using thalidomide in as low a dose as possible.

Our conclusion: in selected patients under careful supervision thalidomide is a valuable addition to our therapeutic armament.

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Immunocompetent cells in psoriasis

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Immunocompetent cells from exacerbating untreated psoriatic skin lesions were immunophenotypically studied by the application of a selection of monoclonal antibodies in a two-stage immunoperoxidase technique. Epidermal changes included (1) focal accumulation of immunoglobulins in the stratum corneum as demonstrated by a mixture of monoclonal anti-kappa and anti-lambda antibodies, (2) focal accumulation of OKM-1 positive but Mo-2 negative cells high in the epidermis, reflecting granulocytes in Munro's abscesses, (3) a marked decrease in epidermal Langerhans cells with focal abnormal clumping and smaller dendrites, as demonstrated by monoclonal anti-HLA-DR and anti-T6 (OKT-6) antibodies, (4) sporadic exocytosis of mainly T1 (Leu-1), T8 (Leu-2a) positive suppressor/cytotoxic T-lymphocytes.

The dermal infiltrates were found to consist mainly of partially activated T1 (Leu-1), T4 (Leu 3a) positive T-helper/inducer cells with a smaller proportion of T1 (Leu-1), T8 (Leu 2a) positive suppressor/cytotoxic lymphocytes. These cells were found to be in close apposition to T6 (OKT-6), HLA-DR positive Langerhans cells and were further accompanied by a few OKM-1, Mo-2 positive monocytes. No B-cells or plasma cells could be demonstrated in the dermis. Human natural killer cells were observed only sporadically.

These results fit best with the hypothesis that psoriasis is a chronic inflammatory condition resulting from persistent stimulation of T cells by immunogen(s) of epidermal origin. However, some observations need further clarification.

A neglected side-effect of PUVA therapy: hypertrichosis

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According to most authors the occurrence of hypertrichosis after PUVA therapy is relatively rare. We have studied two groups of female patients: one category received PUVA therapy (mainly for psoriasis), the second group received UV-A therapy without psoralens (mainly for vitiligo). It was noticed that moderate to severe hypertrichosis developed in the majority of cases in the PUVA-treated group (11 of 14, 79%). Hypertrichosis was most marked on the face and extremities, less so on the trunk. Excessive hair growth started within 6 to 12 weeks after the commencement of PUVA therapy. In the UV-A treated group hypertrichosis was exceptional (1 of 9, 11%). We conclude that hypertrichosis is a common side-effect of PUVA therapy. The hair growth can most probably be attributed to the psoralens, although UV-A light may play a potentiating role. Although the severity of the underlying disease necessitating PUVA therapy usually outweighs the cosmetic problem of hypertrichosis, we feel that patients should be informed that this phenomenon may arise during treatment.

SUMMARIES OF CLINICAL POSTERS

Muco-cutaneous lymph node syndrome (MCLS) of Kawasaki

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History. Male aged 10 months. The disease started with fever (40°C) and listlessness. Two days later a rash appeared on the buttocks, palms and soles. Admission to a community hospital ensued. Soon after admission the rash spread, especially over the trunk. Conjunctival congestion developed. Dryness and fissuring of the lips were also noticed. The patient had mild diarrhoea and there was a vague suggestion of meningism. A diagnosis of Kawasaki disease was made and, on the 5th day of the disease, the patient was referred to our hospital.

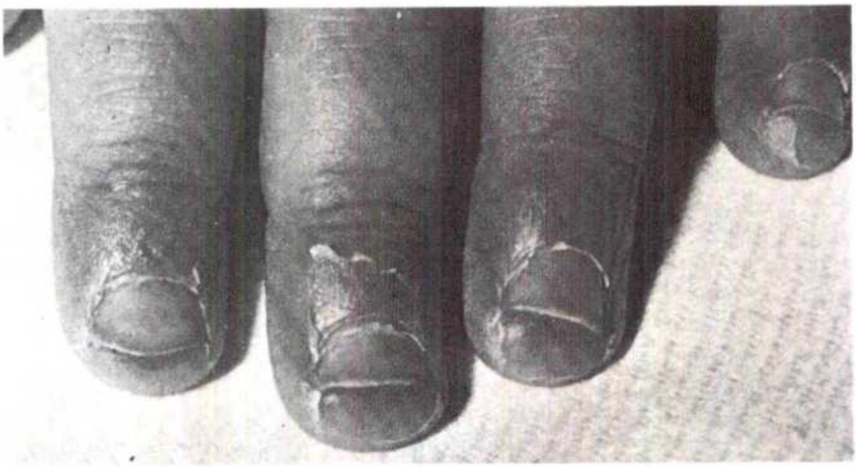

FIGURE 2. Characteristic scaling of fingertips in Kawasaki's disease.

Physical examination. The patient fulfilled all six principal criteria of Kawasaki disease (at least five items are necessary for diagnosis of MCLS; guidelines of the Japan MCLS Research Committee, 1980): fever; bilateral congestion of conjunctivae; dryness and fissuring of the lips, strawberry tongue, diffuse reddening of oral mucosae; reddening of palms and soles followed by desquamation in the convalescent period (Fig. 2); exanthema, mainly on the trunk, without vesicle formation; cervical lymphadenopathy. Moreover, there was mild diarrhoea. Minimal signs of meningism were also present. There were no features of carditis or arthritis.

Laboratory. The following abnormal findings are consistent with MCLS: (1) increased ESR (30 mm), (2) low haemoglobin level (Hb 6·4 mmol/l), (3) leucocytosis (WBC 13·6 × 10^9/l) with a shift to the left, (4) positive CRP (100 mg/l), (5) increased α_2-globulin (14·4 g/l) and thrombocytosis (340, later 620 × 10^9/l).

Histology. Dilatation of dermal capillaries. Minimal perivascular lymphocytic infiltrations. These findings are non-specific. Immunofluorescence of the skin was negative.

Therapy and follow-up. Before admission to our hospital treatment with amoxycillin and gentamycin was given because of the suspected meningitis. When all cultures proved negative, antibiotic therapy was discontinued. Aspirin was given, 100 mg/kg/day for 1 week after which the dose was gradually decreased to 30 mg/kg/day. The total duration of therapy was 2 months. The clinical picture changed rapidly. By the second week of admission desquamation of fingers and toes was the only remaining symptom.

Discussion. The principal risk of MCLS is cardiac and coronary artery involvement. Numerous aneurysms may be encountered. Approximately 1% of children die because of cardiovascular complications. The aetiology of the disease remains unknown.

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Kaposi's sarcoma in an immune-compromised homosexual

B.Naafs, R.H.Cormane, V.Cejka, J.Van der Lelie, M.F.Prummel, H.Barrowclough and R.J.M.Ten Berge

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History. Male, aged 42 years. The patient was seen for the first time with ulcerations around the anus. These ulcerations were extremely painful. His sexual history revealed frequent anogenital contact. He had suffered twenty-five infections with *Neisseria gonorrhoea* and three with *Treponema pallidum*, which were treated adequately. He felt ill and had recently suffered a severe diarrhoea for which he had been admitted elsewhere. A biopsy was taken from a perianal lesion (1) and a culture for herpes simplex was done. One week later he had to be admitted to the Department of Internal Medicine with severe diarrhoea. In addition, the patient was coughing and was becoming dehydrated.

Physical examination. A sick man with a fever of 39°C was seen. Except from slight dehydration no abnormalities were detected. Around the anus and on the perineum a few erythematous papules were seen, some ulcerating. On the right side of the neck a papule was noticed (Fig. 3) which was oval, slightly reddish-blue, not painful on palpation and felt firm. The surface was smooth and 5 mm in diameter. A biopsy was performed (2).

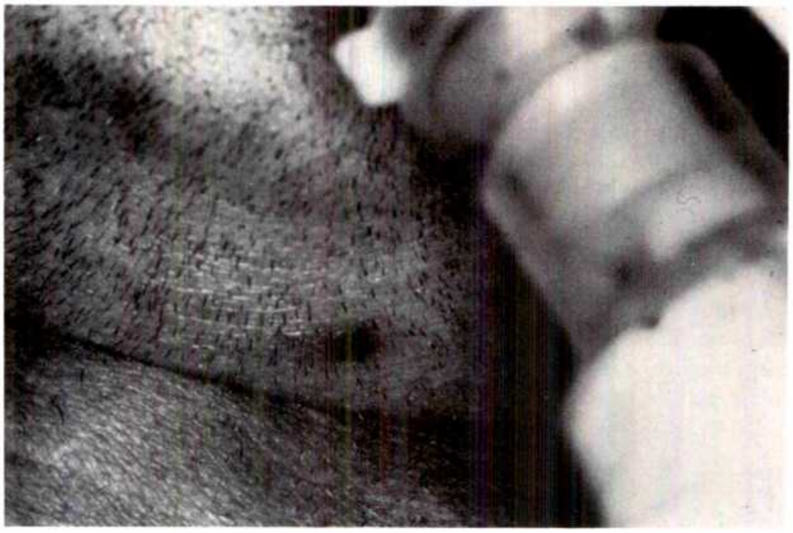

FIGURE 3. Bluish Kaposi papule on the right side of the neck in intubated patient.

Laboratory. ESR 60 mm/h, Hb 7·7 mmol/l, WBC $4{\cdot}7 \times 10^9$/l with only 5% lymphocytes. The T helper/T suppressor cell ratio was reversed. Further investigation showed no abnormalities.

Histology

(1) An epidermis with vesiculation and swollen multinucleated cells was seen. There were swollen endothelial cells in the dermis. One of them had inclusions in the nucleus. Diagnosis: *Herpes simplex*. The possibility of a concomitant cytomegalovirus infection was considered.

(2) A normal epidermis with a diffuse proliferation of endothelial cells in the dermis was seen. There were newly formed blood vessels and poorly outlined vascular spaces and around them there were spindle-shaped cells showing nuclear pleomorphism. Diagnosis: Kaposi's sarcoma.

Follow-up. During admission the patient became severely ill. He showed a Candidosis of the oropharynx, an adenovirus infection of the sigmoid, a *Pneumocystis carinii* infection and a *Herpes* infection of the lungs. *Cytomegalovirus* could be cultured from the urine. Later a *Proteus mirabilis* sepsis developed. Many new Kaposi lesions appeared. The patient went into shock and died. Post mortem a molluscum contagiosum was found on his shoulder.

Discussion. Increasing numbers of Kaposi's sarcoma in immune-compromised patients are being reported (Purack, 1982; Myskowski, Romano & Safai, 1982). This is a report of the first Dutch patient.

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Sarcoidosis

J.D.Bos and D.Tio

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History. Male, aged 26 years. For the past year the patient has had a skin condition consisting of slowly enlarging non-itching dark spots which have been increasing in number. The patient was born in South America and had never been ill. He was not using any medication. There were no other complaints, especially not of general malaise.

Physical examination. There was a disseminated eruption, especially on the limbs, consisting of hyperpigmented, sometimes erythematous, papules and nodules. Some of them showed on diascopy an apple-jelly-like appearance. Also, periocular and perioral, small pin-head sized, skin-coloured papules were apparent (Fig. 4). General examination revealed hepatosplenomegaly.

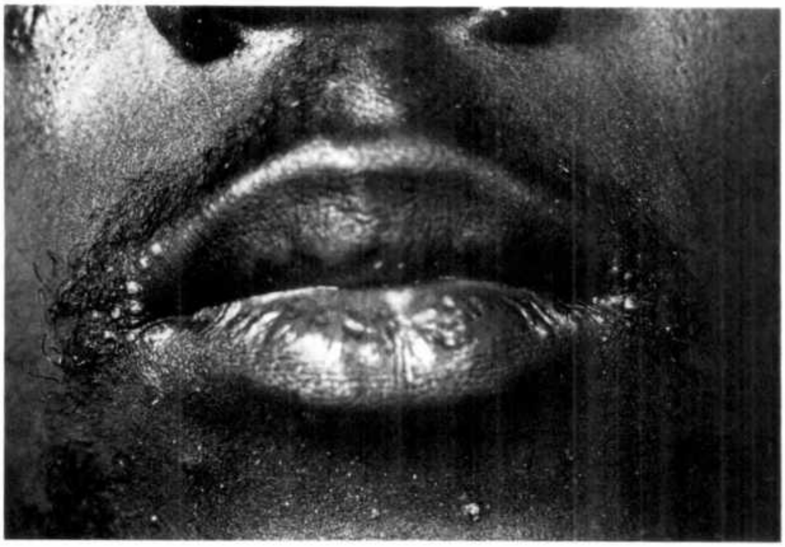

FIGURE 4. Pin-head sized, skin-coloured perioral papules in sarcoidosis.

Laboratory. ESR and haemoglobin: normal. WBC $2{\cdot}8 \times 10^9$/l with normal differentiation. Liver function tests: SGOT 47 U/l, SGPT 69 U/l, LDH 174 U/l, alkaline phosphatase 368 U/l, gamma GT 323 U/l. The creatinine, calcium and phosphate levels were normal. The angiotensin-converting enzyme was slightly increased (65 U/l).

X-rays of the thorax and the hands were normal.

Histology.

(1) Lesion of the back: deep-seated epitheloid cell infiltrates with a small number of lymphocytes and some giant cells were found scattered through the dermis. The small nerves located deep in the dermis were not involved.

(2) Perioral lesion: similar infiltrates but not as deep as in (1).

Diagnosis. Sarcoidosis (cutis).

Discussion. The patient has yet to be investigated for sarcoidosis of the liver and spleen. If the liver and/or spleen prove to be involved, then this patient must have generalized sarcoidosis with an unusual clinical presentation, since there is no apparent pulmonary involvement. Until now however, the patient has refused a liver biopsy, as he has only minimal complaints.

Follicular mucinosis

J.D.BOS AND J.OVERBEKE

Department of Dermatology, University of Amsterdam, and Luthers Diakonessen Ziekenhuis

History. Female aged 53 years. About 6 years ago, the patient discovered slightly itching, red lesions on her right shoulder and, subsequently, a second lesion on the right side of her face. Local treatments were ineffective, but some spontaneous clearing had occurred. She was otherwise healthy and did not use any medication.

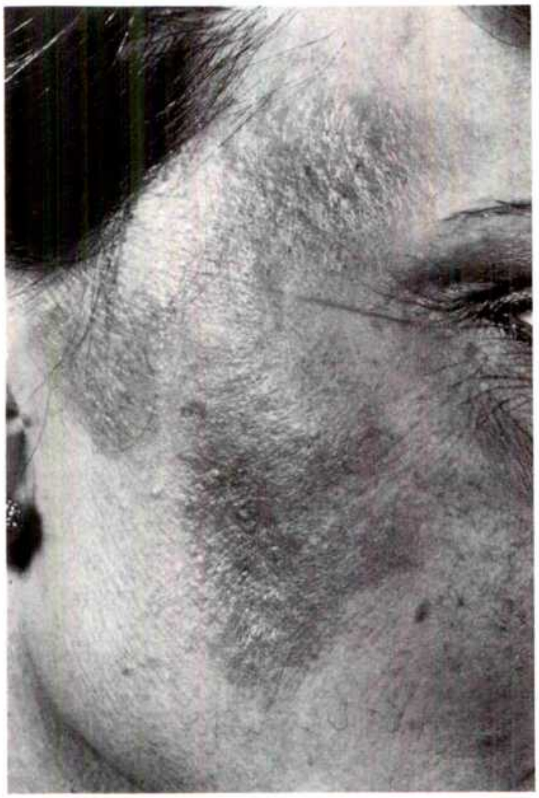

FIGURE 5. Follicular mucinosis.

Physical examination. On the right side of the face (Fig. 5) and on the right shoulder there was a large partially indurated, polycyclic, somewhat brown but otherwise erythematous plaque with follicular papules. On diascopy there was no indication of a granulomatous disease.

Histology. With haematoxylin and eosin staining the epithelial cells of the follicle showed vacuolization

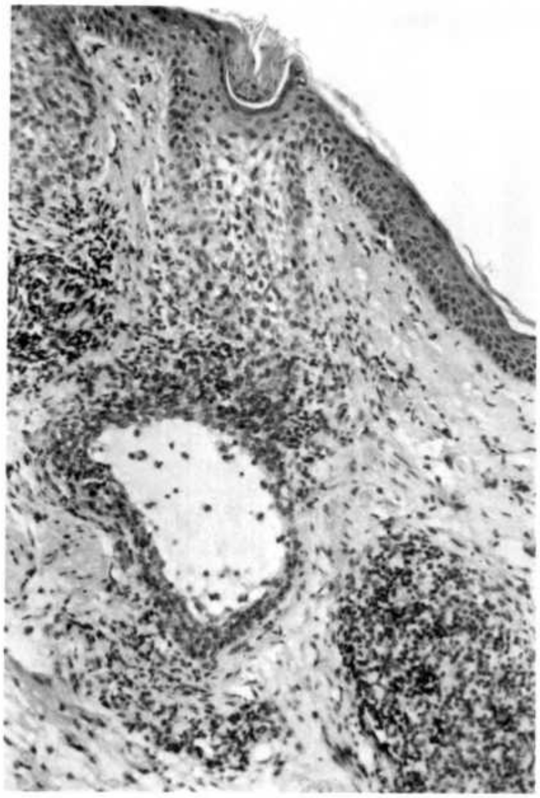

FIGURE 6. Follicular changes in follicular mucinosis.

and intercellular oedema or deposition with a tendency to vesicle formation (Fig. 6). The deposition between these cells and also in the cells themselves, was PAS-negative, but showed mucoid material which was Alcian Blue positive. Perifollicular localized lymphoid cells were present. There were no Pautrier's abscesses.

Discussion. Follicular mucinosis (alopecia mucinosa) is a rare condition of unknown aetiology. It occurs as a primary disease but may also be seen secondarily in mycosis fungoides. For this reason a nuclear contour index and DNA cytophotometry of the lymphoid cell infiltrate were determined and found to be normal. Treatment modalities for the primary form may include chloroquine, superficial radiotherapy and dapsone. Our patient was treated with 100 mg dapsone daily without effect.

Neonatal subcutaneous fat necrosis with hypercalcaemia

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Departments of Dermatology and Paediatrics, University of Amsterdam

History. Male, aged 4 weeks. The patient had been delivered by caesarean section because the mother had a fever, ruptured membranes, foul-smelling amniotic fluid and because there was fetal tachycardia. His birth weight was 4210 g and the Apgar score was 2 after 1 min, and 7 after 10 min. The baby was treated

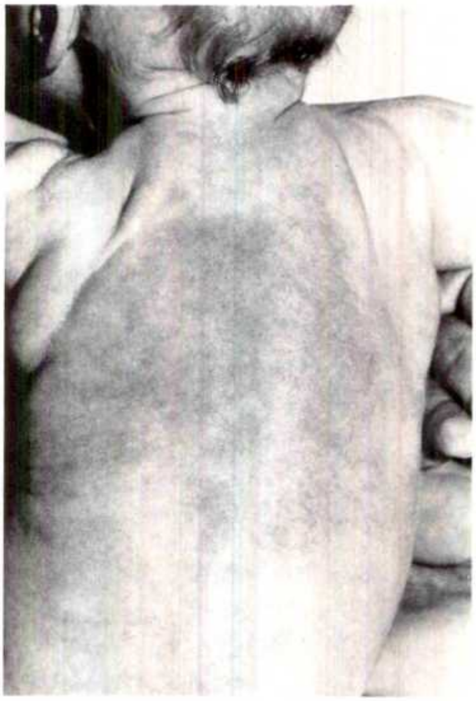

FIGURE 7. Erythematous indurated slightly tender plaques.

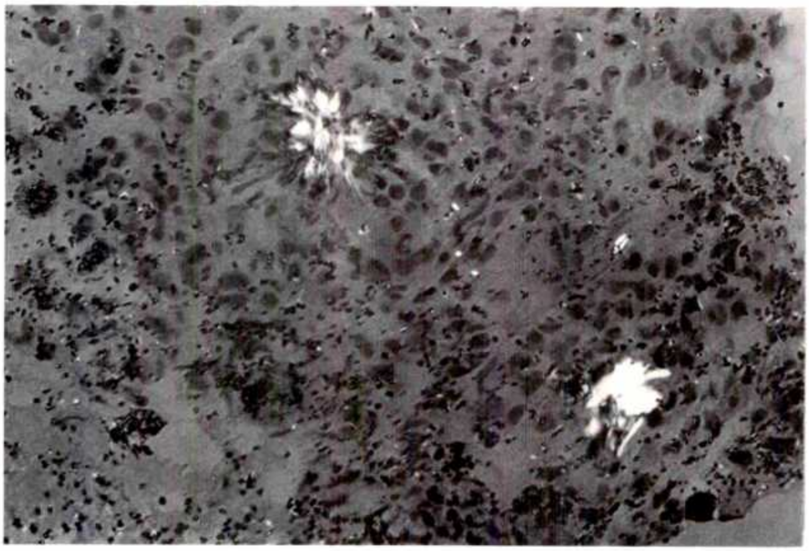

FIGURE 8. Skin biopsy. Subcutis showing necrotic fat cells and a dense infiltrate of histiocytes, lymphocytes and foreign body giant cells filled with needle shaped crystals (doubly refractile). (Haematoxylin & Eosin, × 230.)

with ampicillin and gentamycin since septicaemia was suspected. After 7 days the first skin symptoms appeared. At 4 weeks of age the baby was admitted to hospital because of failure to thrive.

Physical examination. A moderately ill-looking baby weighing 4000 g was seen. Systemic examination was normal. The skin, however, showed multiple erythematous indurated subcutaneous nodules, grouped together to form large plaques (Fig. 7). They were mainly located on the back and shoulders. Some lesions seemed painful on palpation.

Laboratory. The results were unremarkable.

Histology. Normal epidermis and dermis. In the superficial part of the subcutis broad bands of connective tissue were seen. In the deeper part there was degeneration, necrosis and crystallization of fat. An inflammatory infiltrate was seen consisting of histiocytes, lymphocytes, granulocytes and foreign body giant cells. There were needle-shaped crystals localized within the fat cells and the foreign body giant cells (Fig. 8).

Follow-up. During the first 5 days after admission a dramatic rise in the serum calcium was seen (from 2·24 mmol/l to 5·30 mmol/l). Prednisone, in an initial dose of 12 mg/day, together with a calcium-poor and vitamin D-free diet, was started. The hypercalcaemia and the skin lesions subsided rapidly under this regimen. Within 2 weeks the prednisone could be tapered off to zero without recurrence of the lesions and the hypercalcaemia. The child was discharged on a diet low in calcium and vitamin D without any sequelae.

British Journal of Dermatology (1983) **109**, 489–493.

Correspondence

Cryosurgery in cutaneous leishmaniasis

SIR, We read with interest the recent report by Bassiouny *et al.* (*British Journal of Dermatology* 1982, **107**, 467) in which they advocate cryotherapy as an effective treatment for cutaneous leishmaniasis. There has been no similar report for over 70 years (Broome, 1911) and there is a great need for a simple, once-only treatment for cutaneous leishmaniasis, which normally occurs in areas where hospitalization and courses of systemic drugs are inappropriate. If substantiated by formal trials, cryotherapy could prove to be very useful.

We are, however, also concerned by several points in Dr Bassiouny's paper, especially by their unsubstantiated and dangerous statement that 'Adequate cryotreatment of cutaneous leishmaniasis will preclude the development of mucocutaneous extension . . . '. We presume that they are referring to South American cutaneous leishmaniasis due to *Leishmania braziliensis braziliensis*.

As the latent period between the onset of the cutaneous and mucosal lesions in mucocutaneous leishmaniasis can vary from a few months to 24 years (Walton, Chinel & Equia, 1973) and as less than 10% of cases have active skin lesions at the time of mucosal involvement, it would seem likely that there is early systemic spread of the infection to mucosal surfaces where the *Leishmania* can remain undetected for long periods of time. Local therapy of an established skin lesion caused by *L. braziliensis* is most unlikely to affect organisms already disseminated and the absence of *Leishmania* in the primary skin lesion is no guarantee that late mucosal involvement will not occur.

To quote Marsden *et al.* (1977), 'It follows, therefore, that to a certain extent, a decision regarding therapy will be governed by the type of leishmaniasis present in the area . . . '. We would like to state more firmly that in some areas the identification of the infecting *Leishmania* is essential if correct therapy is to be administered. To rely solely on established data relating to the types of *Leishmania* likely to be encountered in a given endemic area can also lead to possible inappropriate therapy. Since 1978, 137 cases of cutaneous leishmaniasis, acquired in Belize, have been treated at the Cambridge Military Hospital in Aldershot. Since April 1981, forty-six new cases have been seen and positive cultures have been obtained from twenty-eight of them. Twenty-three of these cultures have been identified as *L. braziliensis braziliensis* – a parasite not previously reported north of Brazil (Peters, Evans & Lanham, 1983). Cryotherapy or other forms of local treatment would be inappropriate for these patients, whose disease was originally presumed to have been due to *L. mexicana mexicana*. In the Old World there is increasing evidence that the three cutaneous parasites *L. major*, *L. tropica* and *L. aethiopica* elicit immune responses that differ in efficacy. For example, only *L. aethiopica* is capable of causing diffuse cutaneous leishmaniasis. Infections caused by these parasites differ in their response to antimonials. Parasite typing is likely to be useful in studies to determine the best treatment for each species.

There are several other features of Dr Bassiouny's paper that concern us. In his arguments against the use of systemic antimony he misquotes one of us (Bryceson, 1970) who used the drug in prolonged high dosage for the refractory diffuse cutaneous leishmaniasis. The disease was the cause of scarring, not the antimonial. He implies that subzero temperatures selectively kill *Leishmania*, not host cells, and uses the term thermosensitive to describe this phenomenon, quoting papers on heat treatment in support. More serious is the omission of any untreated control subjects in his report, which will be necessary in future studies designed to establish the efficacy of cryotherapy for treating cutaneous leishmaniasis due to *L. major*, a condition that normally heals spontaneously in around 6 months.

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Merkel cell tumour

SIR, I enjoyed reading the case report of a Merkel cell tumour by Dr Rustin and his colleagues (*British Journal of Dermatology* 1983, **108**, 711).

The case for including Merkel cells in the APUD cell series has been strengthened by the demonstration of the APUD cell marker enzyme neurone-specific enolase (Schmechel, Marangos & Brightman, 1978), in the cytoplasm of normal mammalian cells (Gu *et al.* 1981), and in the cells of Merkel cell carcinomas (Kirkham & Isaacson, 1983; Wick *et al.*, 1983).

The problems of differential diagnosis posed by this tumour can be minimized by an awareness of the characteristic history of a slowly growing apparently angiomatous tumour in an elderly patient which has usually been present for less than a year and by having well-fixed biopsy material for histological examination (Kirkham & Cole, 1983).

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Natural killer cells involved in lymphomatoid granulomatosis

SIR, Using a panel of monoclonal antibodies (McAb) defining immunocompetent cell subsets, we were able to study the immunophenotype of the cells in a skin granuloma from a patient with lymphomatoid granulomatosis.

A 36-year-old woman presented with a slowly progressive eruption consisting of numerous erythematous nodules (3 cm diameter), about 1 month after her return from a journey to Australia and Singapore. Subsequently she developed headache and an undulating low fever, later accompanied by disturbed vision. Apart from the rash, general examination revealed only a slightly enlarged spleen. An X-ray of the chest showed a solitary nodule in the left lower posterior lung segment (5 cm diameter). Extensive laboratory investigations revealed a slight anaemia with abnormal lymphocytes in the blood. The T4/T8 ratio was found to be reversed (0·17). Neither serological testing nor cultures revealed any indication of viral infection. Histopathological examination of a skin nodule showed a perivascular dense infiltrate consisting of histiocytes, some with a prominent nucleolus and some double-nucleated, accompanied by a smaller number of lymphoid cells that contained azurophilic granules. On electron microscopy, part of the infiltrate was found to contain lysosomes within lymphocyte-like cells as well as in the histiocytes. On the basis of the clinical and histopathological findings, a diagnosis of lymphomatoid granulomatosis was made. Initial treatment consisted of systemic steroids which returned the patient's temperature to normal.

McAb employed on a fresh-frozen skin biopsy in a two-step immunoperoxidase technique (Bos, Hulsebosch & Krieg, 1983) included: anti-T1 (Leu-1), anti-T4 (Leu-3a), anti-T8 (Leu-2a), anti-HLA-DR, anti-T6 (OKT-6), anti-DRC (a McAb reacting with dendritic reticulum cells, kindly made available by D.Y.Mason, Oxford), anti-HNK-1 (Leu-7), anti-M1 (OKM-1), anti-factor VIII (kindly made available by H.V.Stel, Amsterdam), and anti-kappa/lambda Ig light chains.

The skin granuloma was found to be composed of approximately 10% endothelial cells (anti-factor VIII). Unexpectedly, the cytoplasmic membrane of 30–40% of the cells was found to stain with anti-HNK-1 (Leu-7) (Fig. 1), a McAb reactive with natural killer cells. No T cells, B cells, plasma cells, Langerhans cells or dendritic reticulum cells were observed, and the histiocytes did not stain with anti-M1 (OKM-1).

We had never previously observed such a high proportion of HNK-1 positive cells in any disease studied, including the infiltrates of basal cell carcinoma, cutaneous lupus erythematosus, mycosis fungoides, leprosy, psoriasis and acute disseminated histiocytosis-X.

Although anti-HNK-1 may react with a small percentage of T3-positive and M1-positive cells (Abo,

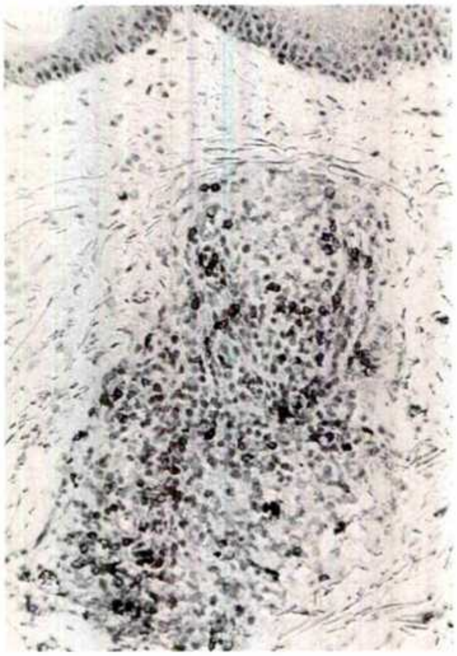

FIGURE 1. Lymphomatoid granulomatosis, showing anti-HNK-1 staining.

Cooper & Balch, 1982), the lack of staining with these McAb in the skin granuloma studied here makes it probable that these NHK-1 positive cells are neither T-cells nor monocytes. The name 'natural killer cells' is still a matter for debate (Klein, 1983): their existence as cytotoxic cells that do not need antigen restriction is widely accepted. *In vitro* they have been found to be cytotoxic for virus-infected cells and tumour cell lines (Herberman, Djeu & Kay, 1979), possibly through the recognition of the transferrin receptor (Vodinelich, Sutherland & Schneider, 1983). We suggest that in our case of lymphomatoid granulomatosis, natural killer cells are reacting to an uncontrolled histiocytic cell line that might develop into a high grade malignant non-Hodgkin lymphoma.

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Lithium and hair loss

Sir, Two recent letters in the *British Journal of Dermatology* (Dawber & Mortimer, 1982 and Orwin, 1983) have interested us because they suggest that information about lithium and its side-effects is not always readily available. At the Lithium Information Centre we maintain a comprehensive computerized reference file to the lithium literature and we are able to conduct literature searches and provide computer-printed bibliographies for anyone in need of information about lithium treatment (therapeutic uses, side-effects, etc.). For example, our reference file on lithium and hair loss yields thirteen published and twelve unpublished reports and in 1979 we published a review article on the subject (Jefferson *et al.*, 1979). We encourage those who are looking for information about lithium to contact us at the address shown below.

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British Journal of Dermatology (1983) **109,** 494–497.

Book Reviews

Clinical Aspects of Immunology. 4th edn. Edited by P.J.LACHMANN and D.K.PETERS (1982) Oxford: Blackwell Scientific Publications. 2 Volumes. Pp. 1751. Price £90.00.

Clinical Aspects of Immunology has become an 'institution' having originally been introduced by Professors Gell and Coombs in 1963. This is the fourth edition and the book has undergone a marked transformation, with a list of new authors, under the editorship of Professors Lachmann and Peters. Not all the changes are necessarily for the better, but it still remains an immensely important text. Though the number of pages has hardly changed, it is now in two volumes, and the format has allowed the text to be increased twofold; unfortunately the price has increased fourfold!

With very major advances in immunology during the past 10 years, it is immensely difficult to write an all-embracing book on clinical immunology and this edition does not attempt to do so. My two main criticisms of the book as a whole are the fact that the content could have been better balanced, and that it is presented in a somewhat illogical sequence. Thus sections are presented in the following order: (1) Physiological systems of the allergic response, (2) Induction of the allergic response, (3) Advances in immunological technology, (4) Immunological intervention (immunosuppressive and immunopotentiating therapy), (5) Allergic mechanisms of tissue damage, (6) Organ-based immunotherapy, (7) Connective tissue disease, (8) Tumour immunology, (9) Transplantation, (10) Miscellaneous (i.e. cot death and allergy to hymenoptera venoms), (11) Immunology of infections (last, but not least!).

Certain sections will be of particular interest to dermatologists, apart from the chapter on the immunological aspects of the skin diseases, notably the section on the allergic mechanisms of tissue damage. In this section type II and type III hypersensitivity (Coombs & Gell, 1963) have been combined and I am not sure that this is an advance. Type I hypersensitivity (a splendid chapter by T.Platts Mills) warrants over 100 pages of text compared with only 23 pages for type II and III, and 13 pages for type IV. I felt that the chapter on skin diseases was somewhat disappointing, despite the eminence of the author, Dr Irma Gigli. This consists of 22 pages of text and 10 pages of references. A number of conditions are discussed which may (or may not) have an immunological basis; these are, in order, atopic dermatitis, urticaria and angio-oedema, cutaneous vasculitis, bullous diseases, lupus erythematosus of the skin, allergic contact dermatitis and skin diseases associated with inborn deficiencies of the complement system. The accounts of the diseases are factual, but no attempt has been made to group them according to pathogenesis (e.g. hypersensitivity diseases, auto-immune diseases etc). I think that this would have been more worthwhile than listing the diagnostic criteria of atopic dermatitis, or the physical, pharmacological, and biochemical alterations in atopic dermatitis. One small annoyance was the number of spelling mistakes (five in one paragraph on page 791!).

It is very easy to be critical of such a book, but it also has many positive attributes. It is generally easy to read, which is important as it warrants a place on the bookshelf of most clinicians, and the reference sections of most chapters are extremely comprehensive. Although it is not cheap, it is no more expensive than Rook, Wilkinson & Ebling and, bearing in mind that immunological mechanisms play an important role in many skin diseases, I think it is well worth the money.

R.STC.BARNETSON

The Science of Photomedicine. Edited by J.D.REGAN and J.A.PARRISH (1982) New York: Plenum Press. Pp. 658. Price £75.00.

There are a number of books which are required reading for those interested in photodermatology and this new offering, edited by such luminaries as James Regan and John Parrish, might be expected to join this number automatically. However, it may well be too general for this and the preface statement that a 'new science' of photomedicine has developed in the last 20 years, based on molecular photobiology, scientific method and the creative use of physics and sophisticated electro-optical capabilities [*sic*] may well deter.

The first of six sections in the book is devoted to an overview by Parrish which is quite useful. In section two, intended as an introduction to the basic science of photochemistry, Longworth presents an outline of the physical chemistry of the subject which is most refreshing to a biologist. This is followed by S.S.West's discussion of fluorescence in diagnosis, dealing here with the special field of acridine orange fluorochroming and its use in tumour cell diagnosis. Regan, with his colleagues Carrier and Snyder, then provides a general introduction to UV-induced DNA damage and its repair and the section is completed by an excellent discussion by Spikes of the mechanisms involved in oxygen-dependent photosensitization processes (this, of course, includes the actions of the porphyrins). This chapter must surely fulfil the editors' wishes for a concise statement of received knowledge and a pointing of the way. Section three is concerned with normal skin. In the first chapter, Anderson and Parrish put the meat on the bones of their earlier reviews of skin optics and present a good, up-to-date reference. For anyone who wishes to learn about vitamin D and the skin, the next chapter here, due mainly to Holick, should provide definitive reading while Hawk and Parrish provide a reasoned and reasonable introduction to the acute skin reactions to sunlight. However, although the literature reviewed is fair, the chapter has some inaccuracies and the reader is advised to consult the original reference in places. Urbach appears to have found a way to review the rather hackneyed material of photocarcinogenesis in a fresh manner and here again we have a useful reference chapter. The importance of the immune system is similarly well reviewed by Morison and Parrish and this chapter forms a useful introduction to the field. The next section deals with abnormal photosensitivity in the skin, first in relation to drug and chemical photosensitization. Harber, Kochevar and Shalita present a review of current thinking and experimental work in the United States and, if read in conjunction with the chapters by Spikes and by Morison and Parrish, this gives a very reasonable picture of this complex field. The unenviable task of reviewing the photodermatoses within the context of this book has fallen to Helmut Ippen, a task made no easier by being in a foreign language. What has evolved is a rather individual, one might say Ippenesque treatment of the subject matter which probably serves a purpose in indicating where the 'scientific method' might be applied with benefit but also widens the reference field with advantage. There follows a section on photoprotection in which Krinsky describes the mechanisms of carotenoid protection against photosensitized damage and Matthews-Roth discusses its application in the photodermatoses. Pathak, Fitzpatrick and Parrish complete the sections with standard production on topical and systemic approaches to protection.

The final section is concerned with phototherapy and photochemotherapy in their various forms and appears to be organized in a rather haphazard way. Next some potted reviews of photodynamic inactivation of herpes virus, dye–light therapy for viral, bacterial and fungal infections; transforming activity of viruses after dye–light inactivation; photochemotherapy of skin diseases and finally, photoradiation therapy of human tumours. These chapters do, at least, provide an introduction to the special parts of the field with which they are concerned.

Overall, the book probably does not come up to the expectations of the editors. Much of the material is already available in other publications, so that it is difficult to recommend *The Science of Photomedicine* without reservation (for dermatologists, the special issue of the *Journal of Investigative Dermatology*, Vol. 77, No. 1, 1981 may be as useful). Nonetheless, it does form a useful reference addition to the specialist photodermatology library.

B.E.JOHNSON

Essential Fatty Acids and Prostaglandins. Progress in lipid research. Vol. 20. Edited by RALPH T. HOLMAN (1982) Oxford: Pergamon Press. Pp. 911. Price £56.00.

The unfortunate story of benoxaprofen, and the tantalising glimpses it yields of possible efficacy in psoriasis, has prompted widespread interest or even active research involvement in the skin pharmacology of leukotrienes and prostaglandins. The appearance of this book, which comprises the collected proceedings of the Washington International Congress on essential fatty acids and prostaglandins is thus dermatologically timely. Because of rapid developments, the contents are already badly dated. However, the book contains contributions from most of the big names in this field. The reviews of Bengt Samuelsson and Howard Morris provide an excellent perspective on the history of SRS-A and the leukotrienes. It is also interesting to see how the same idea (the common identity of the peptidolipid leukotrienes and SRS-A) appears to have germinated simultaneously in the minds of independent groups who nevertheless used different experimental approaches to achieve the same result. The book contains sections on the biosynthesis of prostaglandins, nutritional aspects of essential fatty acids, cardiovascular disease, platelet function, cancer, anaphylaxis, inflammation, immunity and leukocyte function. There is also an allergy/skin section but disappointingly this contains only one dermatological contribution, from Ziboh of Miami who describes a mouse model for psoriasiform epidermoproliferative disorders. It is induced by topical application of eicosatrienoic acid, a fatty acid which interferes with the transformation of arachidonic acid to prostaglandins. Unfortunately, the effects on the lowered skin PGE_2 levels and on epidermal kinetics and scaliness of administration of PGE_2 are not described. However, material of dermatological relevance can be found elsewhere. Zurier's study of the inhibitory action of evening primrose oil on polymorphonuclear chemotaxis and on adjuvant arthritis are of interest because of recently proposed beneficial actions of this agent in atopic dermatitis. The same oil also appears to protect genetically predisposed mice from developing lupus erythematosus. The book is well indexed and clearly a useful source of references for those already engaged in research in this area. Because of its 'how we got where we are' chapters, it is probably also a reasonable acquisition for those proposing to dip a researching toe into the murky waters of prostaglandin research. However, those more general readers who simply wish to keep abreast of developments of dermatological relevance would do better to follow the proceedings of the S.I.D., E.S.D.R. and B.S.I.D. over the next year or so.

M.W.GREAVES

Neonatal Skin – Structure and Function. Edited by H.I.MAIBACH and E.K.BOISITS (1982) New York: Marcel Dekker. Pp. 296. Price S Fr. 118.

A book on the structure and function of neonatal skin is long overdue. This one has been well worth waiting for, as Maibach and Boisits have collected together a number of excellent contributions from clinical dermatology and the pharmaceutical and cosmetic industry. The book is divided into a short introduction on comparative structure of infant and adult skin, which brings together much valuable information. The section on adult and neonatal sweating is also of considerable interest, although one is surprised to see no mention of the work of Verbov on the onset of sweating in early infancy. The observations on the neonatal heroin-withdrawal syndrome as a possible explanation of the neonatal sweating handicap is information that somehow could only have come from Western America. It is the section on the function of neonatal skin which I found most valuable. It starts with a review of the modern techniques measuring transepidermal water loss and continues with further sections on skin barrier function, accenting the increase in transepidermal water loss in premature infants of gestational age of up to 28 weeks. Such information has enabled the neonatologist to understand a great deal more of the problems of the very premature neonate, especially those of water and electrolyte balance and thermal control. Gaseous exchange across the skin of the neonate is also discussed in various short sections. In the section on percutaneous absorption the dangers of systemic toxicity from local applications such as hexachlorophene and iodine in the very young neonate are demonstrated; this has been amply confirmed by other workers in this country especially Rutter from Nottingham. The section on the *in vitro* comparison of adult versus neonatal skin also contains much valuable information again demonstrating the relative vulnerability of the neonate to the penetration of agents which may be toxic on systemic absorption.

There are further sections on the bacteriology of newborn skin and several valuable chapters on diaper dermatitis. Indeed these chapters are probably the best scientific analysis of problems occurring in this area that the reviewer has seen. The book is drawn to a close by an excellent section on neonatal cutaneous disease by Sidney Hurwitz which discusses many of the transient and more persistent eruptions perhaps only seen by the neonatologist and rarely seen by the clinical dermatologist. The explanation of granuloma gluteale infantum as being due to secondary infection with *Candida albicans* is not one that would be acceptable to paediatric dermatologists in this country

This is an excellent book. It should be in every dermatological and paediatric library, and would be of great interest and value to neonatal paediatricians and those dermatologists with frequent exposure to the neonate.

C.F.H.VICKERS

1982 Year Book of Dermatology. Edited by B.H.Thiers and R.L.DOBSON (1982) Chicago: Year Book Medical Publishers. Pp. 422. Price £31.00.

The *Year Book of Dermatology* is a firmly established tradition. A reviewer barely needs to exhort dermatologists or libraries to buy it. It is a must, for although the average specialist may be familiar with the literature in his or her field of interest, dermatology is now so large a subject that a synopsis of the world outside is essential. Indeed criticism by a reviewer of a book of reviews is almost superfluous. Perhaps it is a pity that the index of authors, previously included in earlier editions, is omitted. It is a perverse fact that one recalls the author but cannot find what heading the subject is listed under, much in the same way as the subject one looks up in the index of one volume of 'Rook' is always in the other. The editorial comment after many of the papers is one of the best features of this book and it continues to be lively and informative and if anything might well be expanded. The review of a particular subject, in this case mycosis fungoides and the Sèzary syndrome, has become an established feature. David Norris and William LeFeber have produced a wealth of information. Practically all of the 330 references are from the last decade and the majority from the last 5 years. The clinician will be familiar with the clinical and therapeutic details and might well disagree with the statement that the combination of electron beam radiation followed by topical nitrogen mustard maintenance therapy are the best treatments for early mycosis fungoides. Photochemotherapy is widely used in the United Kingdom in preference to electron beam radiation and is also used for maintenance is some centres and nitrogen mustard in others. However, a large part of the review is concerned with an overview of the workings of the immune system and the relevance of the abnormal immunological findings in these diseases. This section is to be highly recommended. The *Year Book of Dermatology* is a painstaking arduous undertaking and the authors should be rewarded by the placing of a regular banker's order.

A.DU VIVIER

Cell Wall-Deficient Bacteria: Basic Principles and Clinical Significance. Edited by G.J.DOMINGUE (1982) Reading, Mass., U.S.A.: Addison-Wesley. Pp. 595. 196 figs. Price £16.45.

Cell wall-deficient bacteria have proved difficult to work with, not only in hospital routine laboratories, but also in research. Their importance in disease has become more apparent with time. However, formal proof of their role in some diseases is lacking. The authors have produced a critical and balanced view of a subject which has many contentious areas and, to their credit, do not draw back from giving firm recommendations especially on nomenclature topics. The presentation is excellent with useful and clear figures, many of which are electron micrographs of the microorganisms. The text is highly informative and concise and gives enlightening examples of the problems of isolating these bacteria from clinical material. The chapters, which proceed through the general biology of the bacteria and thence to discussions of various L-forms, have been written by different authors who clearly have first hand experience of the topics. The summaries at the end of each chapter, the reference material and an appendix concerning some practical aspects are especially useful.

Relationships of cell wall-deficient bacteria and Mycoplasmas have been discussed. However, this book is not concerned with Mycoplasmas and concentrates on L-forms of a wide range of bacteria attacking the problem of their relationship to disease.

The editor and authors must be congratulated on producing a valuable contribution to the literature on cell wall-deficient bacteria. I hope that as more information becomes available further editions will appear to help both student and expert.

K.T.HOLLAND

British Journal of Dermatology (1983) **109**, 498.

News and Notices

Archibald Gray Medal 1983

The Archibald Gray Medal for services to British Dermatology has been awarded to Dr Renwick Vickers of Oxford.

Chronic Skin Disease; the NHS, the patient and the community, Manchester, October 1983

A seminar on the above subject will be held jointly with the Dept. of Occupational Health, University of Manchester and the Disabled Living Foundation on Wednesday 19 October 1983 at the University of South Manchester. Further details from Miss M.Dowden, Disabled Living Foundation, 346 Kensington High Street, London W14 8NS (Tel. 01-602 2491).

Paediatric Dermatology Course, Arcachon, April 1984

A course in Paediatric Dermatology will be held at Arcachon, near Bordeaux, France on 24–28 April 1984. Subjects to be discussed include the physiology of the skin of the neonate, and disorders of fibroblasts, lymph vessels and hair. There will be a clinical meeting in Bordeaux. The organizers are Professors M.Larregue and J.Maleville. For further information contact: Secrétariat du Service de Dermatologie, Hôpital des Enfants, 168 cours de l'Argonne, 33077 Bordeaux-Cédex, France.

XIV Annual Meeting of E.S.D.R., Amsterdam, May 1984

The XIV Annual Meeting of the European Society for Dermatological Research (E.S.D.R.) will be held in the Raicongrescentrum, Amsterdam, Holland from 20 to 23 May 1984.

The central theme of this meeting will be: 'Monoclonal antibodies and their application to dermatology'. Invited experts will deliver introductory lectures related to the central theme. There will also be eight workshops. The major part of the meeting will, however, continue with free communications as in past years.

Abstracts should be submitted on the official form. No discrimination will be made by the Programme Committee between abstracts related to the central theme and non-related abstracts. The deadline for submission of abstracts is 15 January 1984. Abstract forms can be obtained from: Herbert Honigsmann, M.D., Secretary – E.S.D.R., Department of Dermatology I, University of Vienna, Alserstrasse 4, A-1090 Vienna, Austria.

World Congress of Tropical Dermatology, Mexico City, October 1984

The Fifth World Congress of Tropical Dermatology will be held in Mexico City, Mexico, 16–20 October 1984, under the direction of Dr R.Ruiz Maldonado. There will be special one-day intensive courses in tropical paediatric dermatology, contact dermatitis and occupational dermatoses. Special lectures and symposia are being organized. There will also be clinicopathological mini-case sessions as well as poster exhibits. Abstracts for consideration for presentation should be typed and double spaced in duplicate. We would like a 300-word abstract for open symposia presentations (15 minutes), a 200-word abstract for free communication presentations (10 minutes), and for clinicopathological mini-cases (5 minutes), a 50-word abstract. The deadline for submission of abstracts for papers and minicases is 31 July 1984. Abstracts should be sent to The Congress Secretary, P.O. Box 101–16, Mexico 04530, D.F.

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Pharmacology and Therapeutics. Up-to-date reports on the treatment of diseases and pharmacology of dermatologically related drugs
Reminiscence a look at the history of dermatology, skin diseases or noteworthy figure in dermatology
Tropical Medicine Rounds irregular reports on some of the special problems encountered by dermatologists working in the tropics
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The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes signed editorials reviewing, primarily for the benefit of the clinician, advances in laboratory research, and also reviews of recent advances in such aspects of dermatology as contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews, correspondence and society proceedings. The Journal is the official organ of the British Association of Dermatologists and also of the Netherlands Association for Dermatology and Venereology, but it attracts contributions from all countries in which sound research is carried out, and its circulation is equally international.

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Manuscripts should be sent to the Editor, Dr J.L.Burton, Dermatology Department, Bristol Royal Infirmary, Bristol BS2 8HW. They should be typewritten on one side of the paper only, with a wide margin, be double spaced, and bear the title of the paper, name and address of each author, together with the name of the hospital laboratory or institution where the work has been carried out. The name and full postal address of the author who will be responsible for reading the proofs should be given on the first page. A duplicate copy, complete with figures, tables and references, should be sent, since in many cases two referees are consulted. The author should keep a carbon copy of his manuscript. The Editorial Board reserves the right to make literary corrections.

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gram(s)	g	minute(s)	min	molar	mol/l
kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3}g)	mg	second(s)	s	gravitational acceleration	***g***
microgram(s) (10^{-6}g)	μg	cubic millimetre(s)	mm^3	micrometre(s)	μm
nanogram(s) (10^{-9}g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12}g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^{131}I
hours(s)	h	milliequivalent	mmol		

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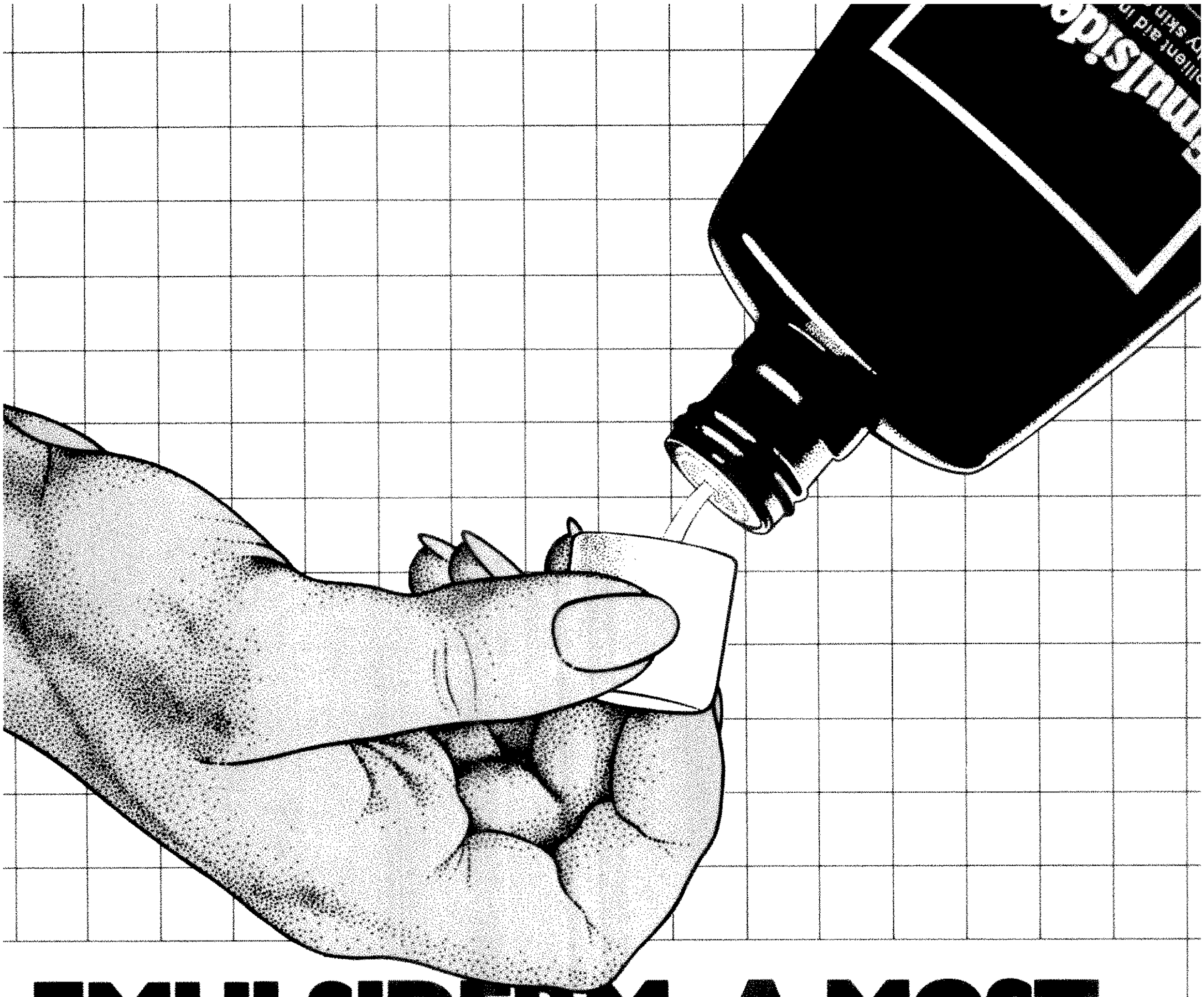

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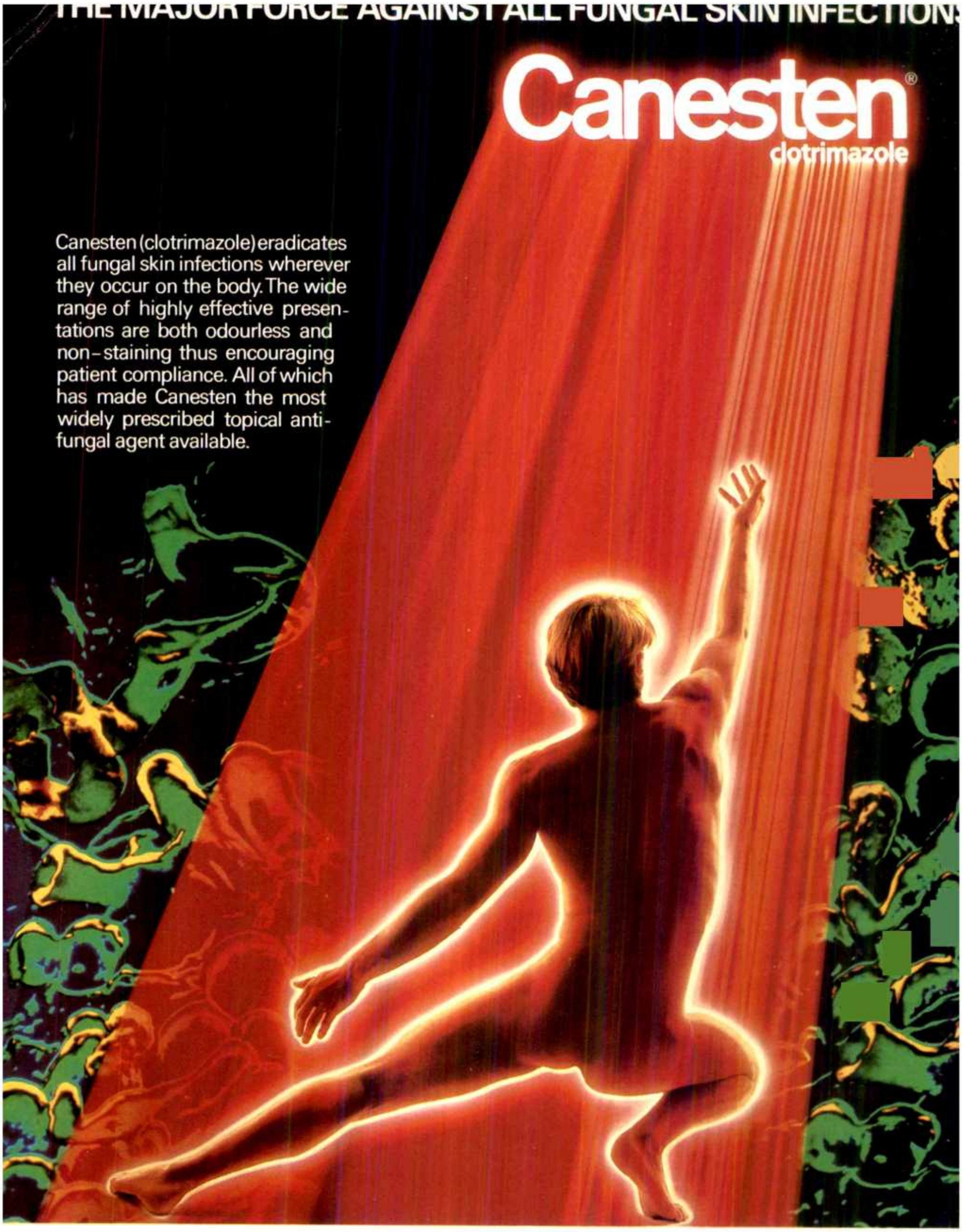

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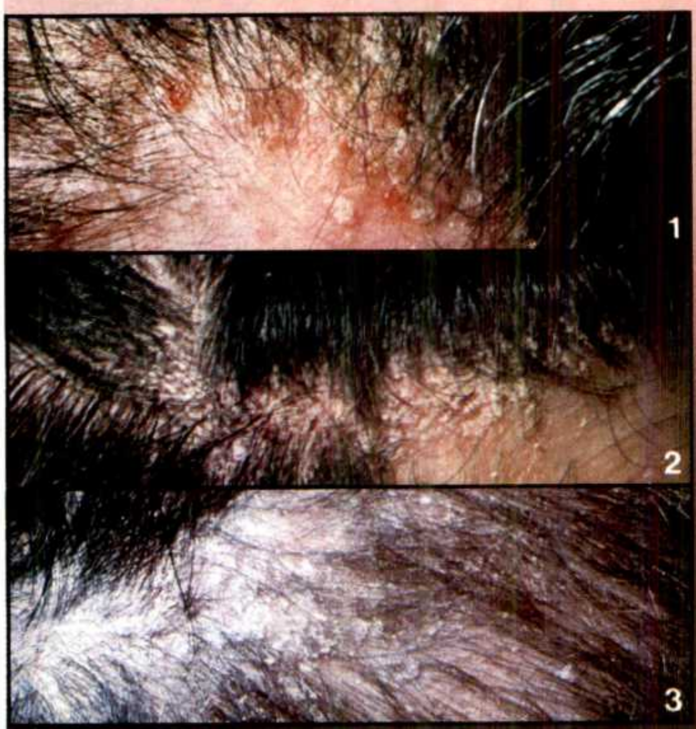

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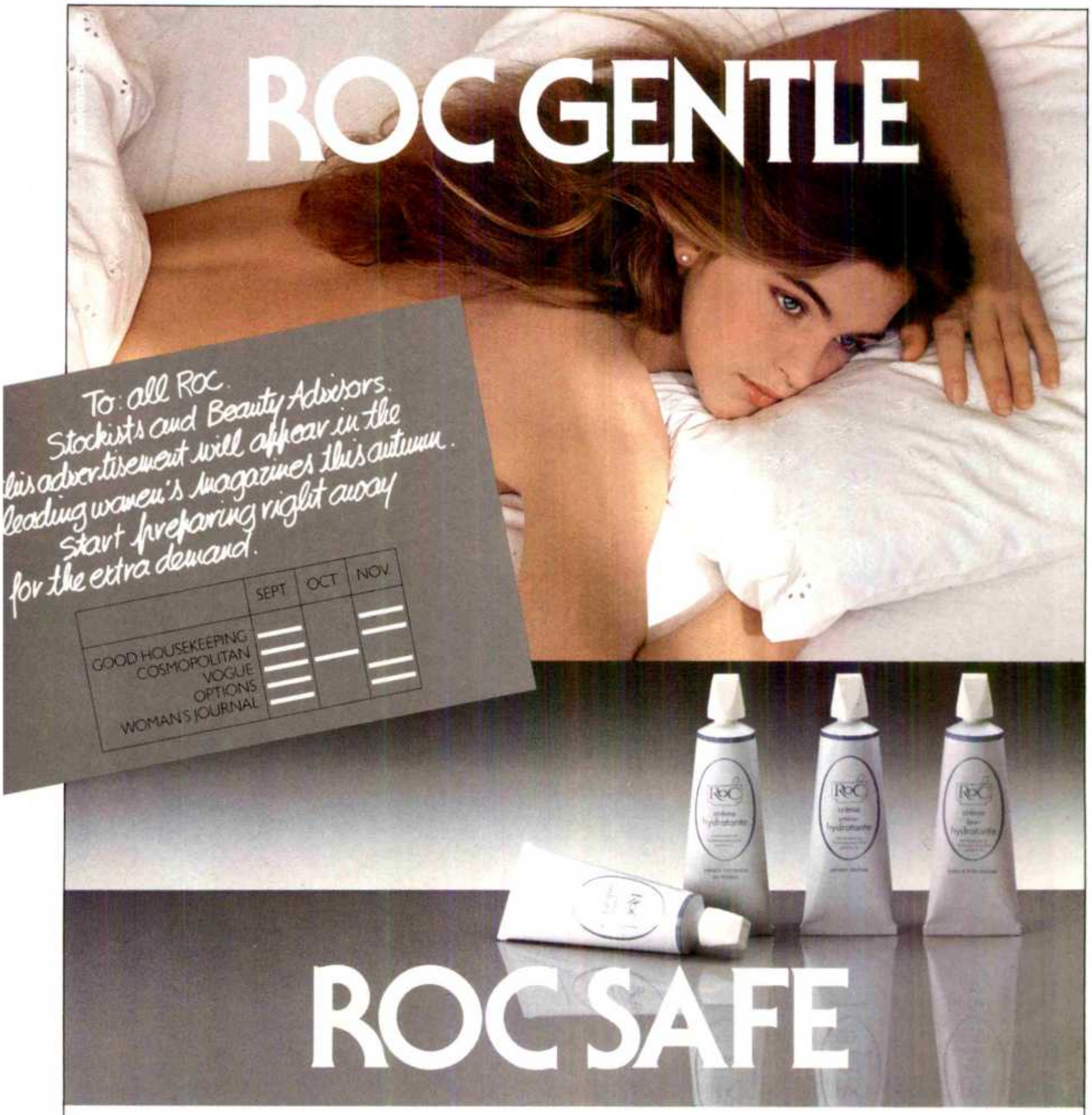

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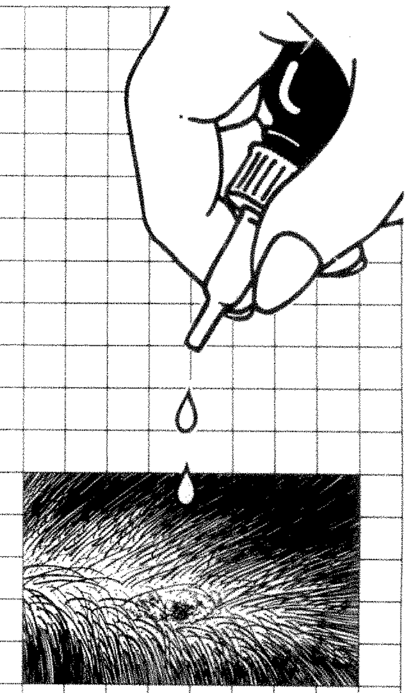

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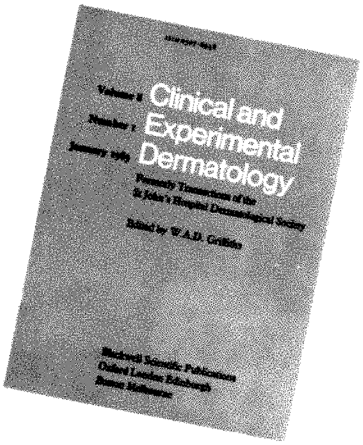

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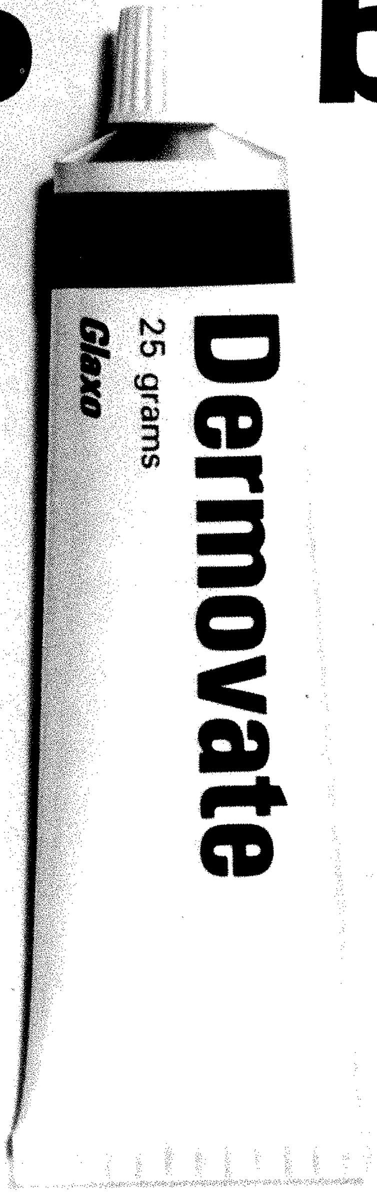

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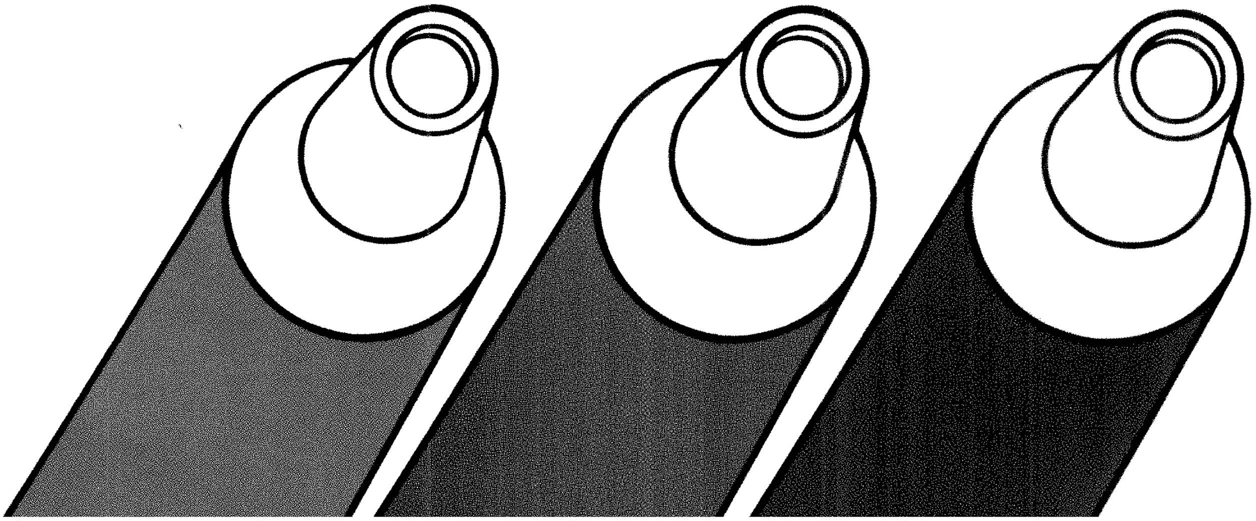

British Journal of Dermatology (1983) **109**, 499–508.

Clinial and Laboratory Investigations

Junctional blisters in acquired bullous disorders of the dermal-epidermal junction zone: role of the lamina lucida as the mechanical *locus minoris resistentiae**

G.F.KLEIN, H.HINTNER, G.SCHULER AND P.FRITSCH

Department of Dermatology, University of Innsbruck, Austria

Accepted for publication 6 April 1983

SUMMARY

The level of cleavage was determined in a variety of acquired bullous diseases of the dermal-epidermal junction zone (bullous pemphigoid, dermatitis herpetiformis, porphyria cutanea tarda and epidermolysis bullosa acquisita). We used an indirect immunofluorescence technique to examine the basal membrane zone with anti-type IV collagen and anti-laminin antisera and bullous pemphigoid sera. The majority of blisters examined proved to be junctional, including those from disorders hitherto considered to be dermolytic. Dermolytic cleavage was encountered only sporadically in microvesicles of dermatitis herpetiformis, in one small vesicle and in one out of five large blisters of porphyria cutanea tarda and in a large lesion of epidermolysis bullosa acquisita. We conclude that in acquired bullous disorders of the dermal-epidermal junction zone the preferential site of split formation is the lamina lucida which appears to act as a *locus minoris resistentiae*; dermolytic split formation of substantial extent occurs only when the sublaminal fibrillar apparatus is mechanically compromised.

The anatomical level of cleavage is currently regarded as a key factor for the classification and diagnosis in bullous skin disorders, based on the tacit assumption that the site of split formation necessarily coincides with the site of action of the major pathological processes involved (Fritsch & Elias, 1979). Being clearly applicable in the distinction between disorders of intraepidermal and 'subepidermal' blister formation, this principle has been extrapolated to the subclassification of blisters of the dermo-epidermal junction zone. Disorders found to produce 'junctional'

---

* Presented, in part, at the Meeting of the European Society of Dermatological Research, Amsterdam, 1982.

Correspondence: Georg F.Klein, M.D., Dept of Dermatology, University of Innsbruck, Anichstr. 35, A-6020 Innsbruck, Austria.

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blisters (i.e. cleavage in the lamina lucida) are thus regarded as fundamentally different from those with 'dermolytic' blisters (i.e. cleavage beneath the basal lamina).

The distinction between junctional and dermolytic cleavage was hitherto dependent on electron microscopy. As an alternative, we recently employed an immunofluorescence technique using antibodies against antigenic determinants of the dermal-epidermal junction zone (bullous pemphigoid serum, anti-type IV collagen and anti-laminin antibodies) (Hintner *et al.*, 1981). This technique, in essence, visualizes the location of the basal lamina within a given blister; as an advantage over electron microscopy, it is rapid and allows the scanning of large specimens, i.e. whole blisters.

When applied to the disease spectrum of hereditary epidermolysis bullosa, the cleavage sites revealed by this technique invariably conformed to the patterns previously amply confirmed by electron microscopy (Hintner *et al.*, 1981). In contrast, this was not the case in the acquired bullous disorders of the junction zone. In this paper, we demonstrate that, contrary to the above tacit assumption, junctional blisters do quite commonly occur in 'dermolytic' bullous disorders. Dermolytic and junctional bullae may arise in immediately adjacent sites and may even represent consecutive stages in the course of pathological events in some bullous disorders.

METHODS

Twenty-four skin biopsies (Table 1) were obtained from sixteen patients with the following disorders: bullous pemphigoid (BP) (five patients); dermatitis herpetiformis (DH) of classical type (five patients); DH of linear type (two patients); porphyria cutanea tarda (Pct) (four patients); acquired epidermolysis bullosa (EBA) (one patient). In all cases the diagnosis had been previously established on clinical, histopathological and immunological grounds. Newly formed blisters (not older than 24 hours) and perilesional skin were excised under local anaesthesia and processed for immunofluorescence (IF). Some biopsies (for details see Table 1) were divided into equal parts and processed for IF and electron microscopy (EM).

Immunofluorescence. Skin specimens were snap-frozen in liquid nitrogen, cut into 4-μm cryostat sections and further processed according to routine indirect immunofluorescence (IIF) procedures. Antibodies against three antigenic determinants of the dermal-epidermal junction zone were used: rabbit anti-type IV collagen (TIV) and laminin antisera (kindly provided by Dr Stephen Katz, NIH, Bethesda, Maryland) and bullous pemphigoid (BP) sera from five different patients with active disease. Normal human and rabbit sera served as controls. For further technical details see Hintner *et al.* (1981).

Evaluation criteria. Bullae were rated junctional if BP antigen was detected at the roof and TIV and laminin at the base of the blister cavity, and dermolytic if all three antigens investigated were located at the blister roof.

Electron microscopy. Skin specimens were minced, fixed (Karnovsky's fixative, 5 h, room temperature), extensively rinsed (0·1 M cacodylate buffer, pH 7·4, 4°C), post-fixed (3% aqueous osmic acid, 4°C), stained *en bloc* with veronal acetate buffered uranyl acetate and, after rapid dehydration in a graded series of alcohols, embedded in Epon 812. Ultrathin sections were cut with Reichert OM U2 and Ultracut® ultramicrotomes, stained with lead citrate and examined with a Philips 400 EM, operating at 80 kV.

TABLE 1. Acquired bullous disorders of the dermal-epidermal junction zone. Correlation of clinical type of lesions to level of cleavage

Diagnosis	No. of biopsies IF/EM	Clinical type of lesion	IIF-findings	
			Type of blister(s)	Level of cleavage
BP	6/2	Blister on normal-appearing skin or inflammatory halo	Single, large	Junctional
	2/0	Urticarial	Multiple, small	Junctional
DH classic	5/3	Large vesicle on erythematous/urticarial base	One large, several small	Junctional
	1/0	Urticarial	Multiple microvescicles	Junctional/ dermolytic
DH linear	2/1	Large vesicle on erythematous base	Large	Junctional
	1/0	Vesicle on urticarial base	One large vesicle, multiple microvesicles	Junctional Junctional/dermolytic
PCT	5/1	Blisters	Large	Junctional (4) Dermolytic (1)
	1/1	Normal-appearing skin close to large blister	Small vesicle	Dermolytic
EBA	1/1	Blister on atrophic, scarred skin	Large	Dermolytic

RESULTS

Bullous pemphigoid. All bullous lesions, regardless of size and presence of inflammatory halo proved clearly junctional. Smaller, more inflammatory lesions exhibited foci of TIV and laminin on the blister roofs in portions corresponding to papillary tips (Fig. 1), and short stretches of BP antigen on the blister base. There was no qualitative difference between the five different BP antisera used. Electron microscopy revealed intact basal laminae on the blister floor on top of the dermal collagen in all lesions studied.

Dermatitis herpetiformis ('classic'). All bullous lesions, regardless of size proved junctional (Fig. 2), with occasional traces of TIV and laminin adhering to the blister roofs (Fig. 3). In an urticarial lesion multiple microvesicles were found. Most of them were junctional, a very few dermolytic (Fig. 4). Ultrastructurally, the basal lamina was found predominantly on the blister floor on top of the dermis (Fig. 5), but in some sections small portions were attached to the basal cells (Fig. 6).

Dermatitis herpetiformis ('linear'). Both large bullae of the first patient were clearly junctional. In some sections patchy traces of TIV and laminin lined the blister roof similar to the

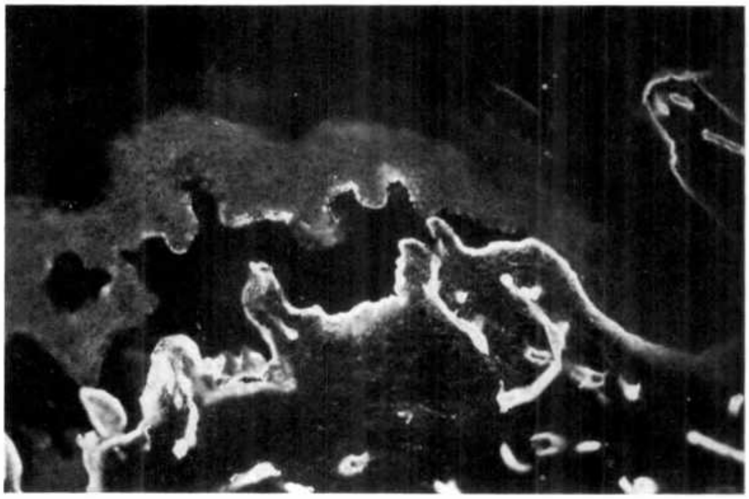

FIGURE 1. Bullous pemphigoid (lateral part of an inflammatory lesion): remnants of TIV in the roof of a junctional bulla. Indirect immunofluorescence (TIV antisera), × 63.

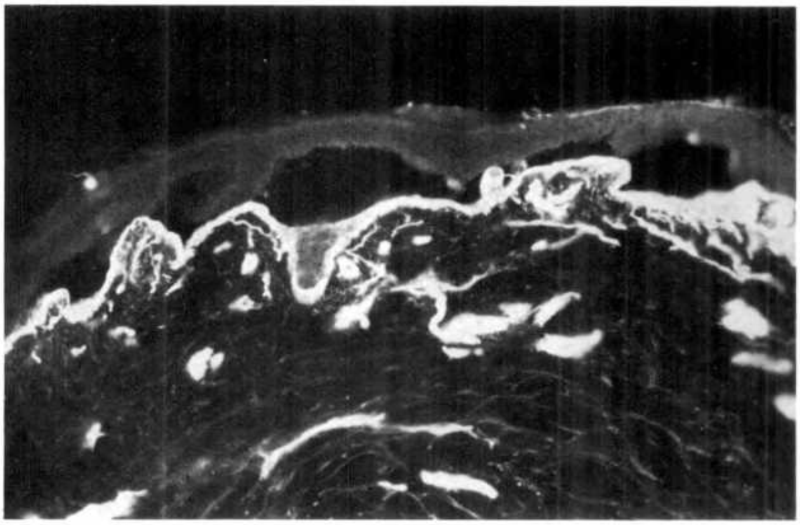

FIGURE 2. Dermatitis herpetiformis (whole large blister): junctional split, indirect immunofluorescence (TIV antisera), × 63.

findings in BP. An urticarial lesion of the second patient exhibited dermolytic microvesicles in the immediate vicinity of larger junctional vesicles. Electron microscopically, the basal lamina was found on top of the dermis in a large bulla.

Porphyria cutanea tarda. One small (Fig. 7) and one large lesion exhibited dermolytic cleavage, whereas four large bullae were clearly junctional (Fig. 8). As a reproducible finding,

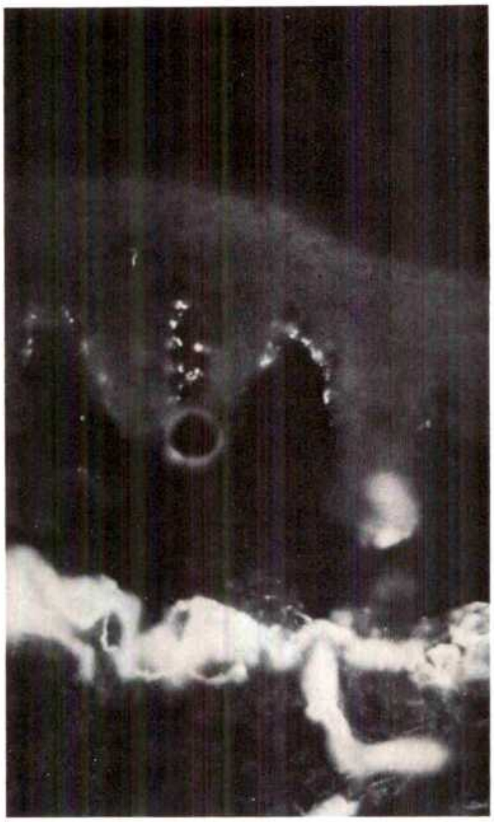

FIGURE 3. Dermatitis herpetiformis: remnants of TIV in the roof of the same junctional blister as shown in Fig. 2 (centre of another section). Indirect immunofluorescence (TIV antisera), × 154.

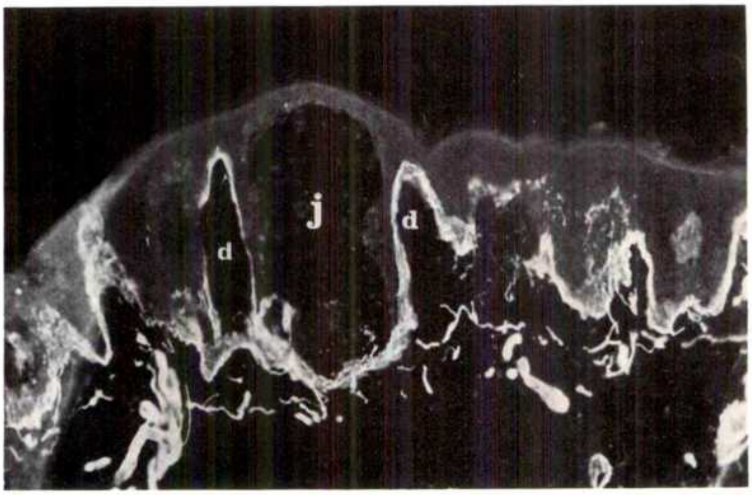

FIGURE 4. Dermatitis herpetiformis (whole urticarial lesion): dermolytic (d) and junctional (j) microvesicles in close proximity. Indirect immunofluorescence (TIV antisera), × 154.

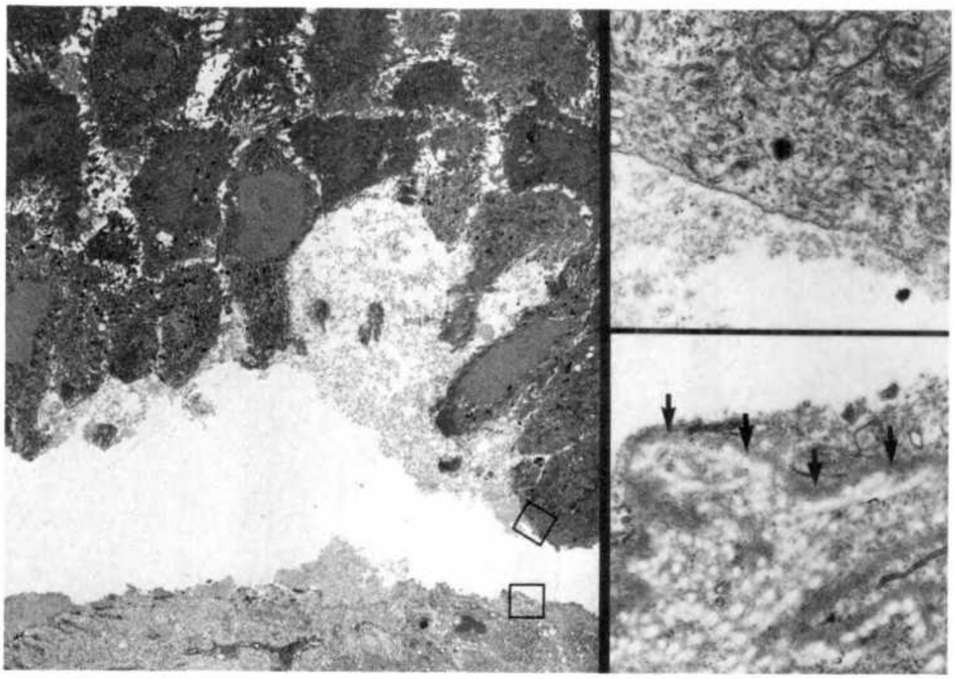

FIGURE 5. Dermatitis herpetiformis (EM): low power view of junctional blister (× 700); higher magnification (× 11,900) of epidermal cell without lamina densa (upper inset) and lamina densa (arrows) on top of the dermis (lower inset) in areas depicted.

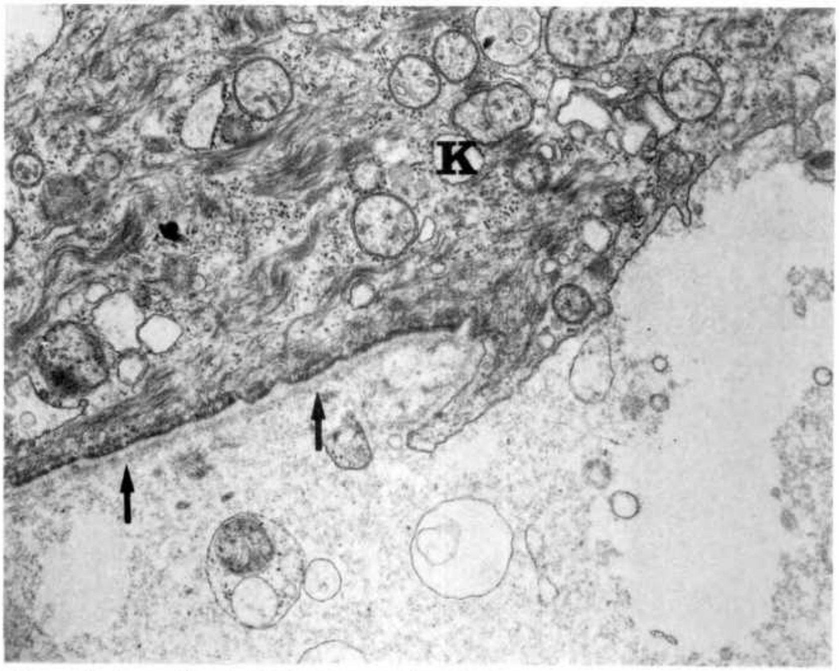

FIGURE 6. Dermatitis herpetiformis (EM): remnant of basal lamina (arrows) attached to keratinocyte (K) in the lesion shown in Figs 2 and 3 (centre of the lesion); × 7000.

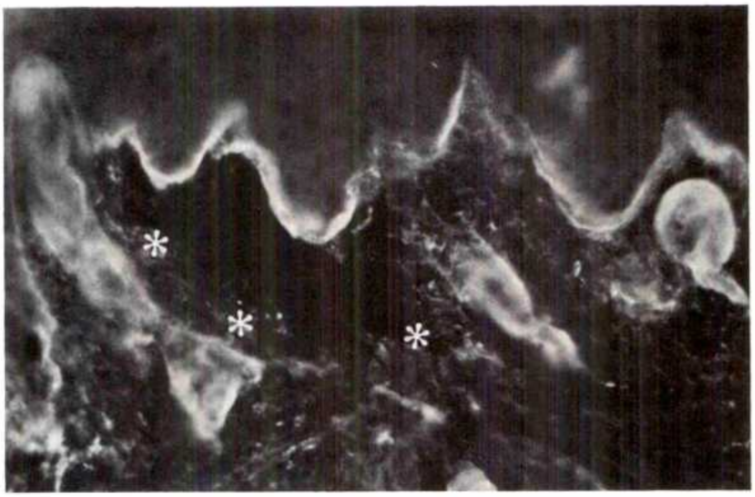

FIGURE 7. Porphyria cutanea tarda: whole dermolytic vesicle; asterisks mark the floor. Indirect immunofluorescence (TIV antisera), × 154.

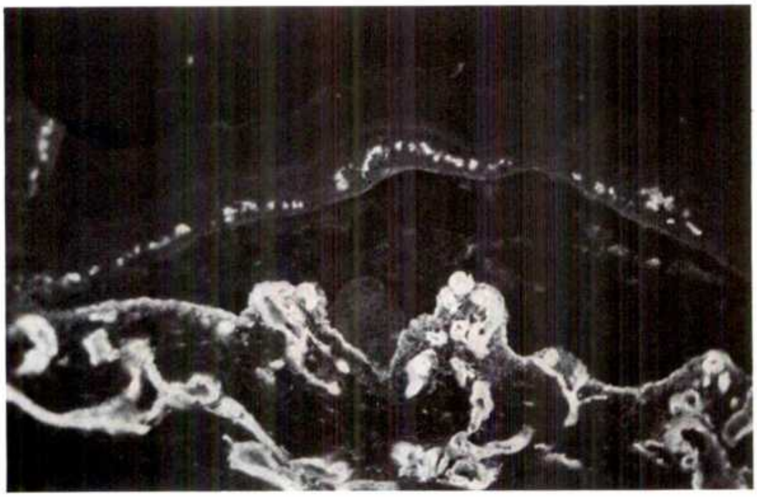

FIGURE 8. Porphyria cutanea tarda: large part of a junctional blister; note patchy linear arrays of fluorescence within the viable epidermis. Indirect immunofluorescence (TIV antisera), × 63.

horizontal patchy linear arrays of TIV and laminin were observed in all lesions examined within the viable epidermis (Fig. 8). These deposits probably represent remnants of basal lamina which had been incorporated into the viable epidermis in the course of regeneration processes.

Ultrastructurally the basal lamina was found on the blister floor on top of the dermis in a large junctional blister whereas parts of it were attached to the basal keratinocytes in the small dermolytic vesicle.

EBA. One single large lesion examined was dermolytic throughout, both at the light and electron microscopic level.

DISCUSSION

The above observations diverge in part from the current views on the level of cleavage in acquired bullous disorders of the junction zone, and challenge its use in diagnosis and classification. The findings reported can be summarized as follows:

(1) Junctional blisters are by far the most frequent type of blister in acquired bullous dermatoses of the junction zone including dermatoses hitherto rated as dermolytic (DH, Pct).

(2) Dermolytic cleavage is only present in microvesicles (DH) or small vesicles (Pct), whereas large vesicles almost invariably prove junctional.

(3) Both dermolytic and junctional cleavage may occur in the same disorder and may even be observed immediately adjacent to each other in the same section.

Although unexpected, these findings can be well reconciled with the current concepts of the mechanisms of vesicle formation. Obviously, vesicles result from fluid influx (due to inflammatory oedema of the papillary dermis as a rule) into skin compartments of impaired mechanical coherence resulting in cleavage. Damage to mechanical coherence may arise from specific damage to target structures by the disease process itself (e.g. acantholysis in pemphigus vulgaris), which then governs the level of split formation of the blister to be formed. If no or only minor damage to the mechanical properties accompanies inflammation, vesicles either do not develop, or they necessarily form in the plane of relatively weakest coherence. It appears logical to assume that the space of the lamina lucida represents such a 'natural' cleavage plane of the skin: it is virtually devoid of fibrillar structures and is the site of a 'viscous bond' between dermis and epidermis (Van der Leun, Lowe & Beerens, 1974) which can be easily disturbed by thermal, mechanical, osmotic and chemical factors (Briggaman & Wheeler, 1975). In contrast, the basal lamina is firmly anchored to the dermal collagen network by the system of the anchoring fibrils and the dermal microfibril bundles. Since the basal lamina is very permeable to water and small molecules, it can be fairly safely assumed that dermolytic cleavage can be expected only in diseases which are associated with damage to the sublaminal fibrillar network. Finally, it can be expected that, in diseases with limited or focal damage to the fibrillar network junctional bullae can arise as a secondary phenomenon on top of dermolytic blisters. This hypothetical explanation appears to be confirmed by our observations.

All BP blisters investigated were clearly junctional confirming previous reports (reviewed by Sams & Gammon, 1982). Complement activating antibodies directed against an antigen of basal cell origin located in the lamina lucida lead to inflammation and fluid influx into the lamina lucida (Gammon *et al.*, 1982) thus impairing the 'viscous bond'. Foci of basal lamina adhering to the blister roof found in urticarial (inflammatory) lesions may be due to occasional ruptures of the basal lamina during the process of blister formation.

In DH, classic type, the microfibrillar bundles of the papillary dermis have been shown to be the target site of IgA antibodies (Yaoita & Katz, 1976; Stingl *et al.*, 1976), which then activate complement via the alternate pathway (Provost & Tomasi, 1974). Inflammation and likely focal fibrillar damage may result in the formation of dermolytic microvesicles. In the absence of gross mechanical damage no larger blister cavities can be found and the dermolytic microvesicles rapidly transform into junctional bullae, which then become the leading type of lesions.

In DH, linear type, both clearly junctional large bullae and dermolytic microvesicles in the immediate vicinity of junctional vesicles may evolve in different patients, probably depending on the localization of the IgA deposits either within the lamina lucida or below the basal lamina (Yaoita, Hertz & Katz, 1976).

How can we explain the fact that the largely junctional nature of the blisters in classic DH has previously escaped recognition by most investigators (Fry & Johnson, 1969; Jakubowitz, Dabrowski & Maciejewski, 1970; Riches *et al.*, 1973; Dabrowski *et al.*, 1977) and has been observed only by Rodrigo (1972)? The reason may be a simple technical sampling phenomenon. Other than in the present study, the previous investigations were exclusively carried out on the electron microscopic level thus allowing only the examination of small sections. For electron microscopy, moreover, only very fresh lesions, i.e. small vesicles or non-vesicular lesions, or even clinically normal skin, are selected as a rule, amounting to a strong sampling bias for dermolytic microvesicles in DH. This interpretation is corroborated by our ultrastructural controls which showed junctional cleavage in deliberately chosen large lesions.

In EBA, the sublaminar regions are occupied by extensive deposits of amorphous granular material of unidentified origin, connected with the disappearance of the anchoring fibrils (Gibbs & Minus, 1975; Hintner, Schuler & Fritsch, 1982; Yaoita *et al.*, 1981). In terms of mechanical impairment, therefore, EBA and dystrophic hereditary epidermolysis bullosa—characterized by the absence of anchoring fibrils—appear to be comparable, leading to clear cut and extensive dermolytic cleavage.

Pct appears to maintain an intermediate position between DH and EBA. Similar to EBA, deposits of amorphous material are found in a sublaminar position around collagen fibres (Epstein, Tuffanelli & Epstein, 1973; Wolff *et al.*, 1982), but the damage to the anchoring system is of much less intensity. It is not surprising, therefore, that both dermolytic and junctional vesicles can be recovered depending on the degree of mechanical impairment in the respective lesion. In Pct, two earlier authors (Pearson, 1967; Wolff *et al.*, 1982) have reported dermolytic cleavage; we assume a similar sampling problem is the underlying cause, as for DH.

The above findings have far-reaching implications both for the understanding of the pathophysiology of blister formation and for the taxonomy of bullous disorders. Junctional bullae may no longer be regarded as a specific morphological feature due to specific pathomechanisms. They obviously may arise either primarily or as a secondary phenomenon consequent to dermolytic blister formation from which they catch the spill-over fluid which breaks or sieves through the basal lamina. The lamina lucida thus appears to represent a mechanical *locus minoris resistentiae* of the junction zone.

ACKNOWLEDGMENTS

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Anti-D47: a monoclonal antibody reacting with the secretory cells of human eccrine sweat glands*

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SUMMARY

Anti-D47 is a monoclonal antibody reacting with a surface antigen of human cortical thymocytes (different from the T6 antigen). It was prepared by immunization of mice with human thymocytes. The known cross-reactivity of monoclonal antibodies prepared against thymic cells with skin components prompted us to test anti-D47 on human skin as well as animal tissues. No labelling was observed on animal tissues. On human skin, anti-D47 was found to react with the secretory portion of eccrine sweat glands (ESG), i.e. with a cytoplasmic antigen of the secretory cells lining the deep portion of ESG glomeruli. No labelling was observed on apocrine sweat glands or on the excretory portion of ESG. Anti-D47 was also tested on histologically proven extramammary (vulvar) Paget's disease and basal cell epitheliomas: no staining of the neoplastic cells was observed. Anti-D47 appears to be an immunological marker of the secretory cells of human ESG and a new tool for the investigation of human sweat gland pathology.

It has recently been shown that cutaneous components share antigenic determinants with cells of the lymphopoietic system, particularly those of thymic origin. This was revealed by the cross-reactivity of monoclonal antibodies prepared against suspensions of human thymic cells with Langerhans cells (e.g. OKT6) (Murphy *et al.*, 1981; Fithian *et al.*, 1981; Chu *et al.*, 1982), or with epidermal cells of the basal cell layer (e.g. BL7, Schmitt *et al.*, 1982). This knowledge prompted us to test a new monoclonal antibody (anti-D47) produced recently against human thymocytes, on human and animal tissues, using an indirect immunofluorescence technique in order to check for cross-reactivity with these tissues.

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METHODS

Preparation of anti-D47

Anti-D47 was prepared according to the method of cellular hybridization (Koehler & Milstein, 1975), by immunizing mice with a suspension of human thymocytes. After selection and cloning anti-D47 appeared to be specific for a surface antigen of cortical thymocytes. The molecule reacting with anti-D47 is a class I antigen of 47,000 molecular weight; it is different from the one defined by monoclonal antibodies NA1 and T6, although they have a very close cellular distribution (Boumsell *et al.*, 1981; Boumsell & Bernard, 1981). The cellular distribution of the antigen defined by OKT6 has been well characterized by Reinherz *et al.* (1980) and has recently been biochemically characterized by Takezaki *et al.* (1982).

Tissue specimens

These included normal human thymus gland, normal facial and axillary skin, biopsies from an extramammary (vulvar) Paget's disease and from basal cell epitheliomas. The latter diagnoses had been confirmed by standard histological examination. Animal tissues studied included: mouse foot-pad skin and thymus gland, and monkey skin, cerebellum, heart and liver.

Immunohistochemical procedure

Tissue specimens were snap frozen in liquid nitrogen, and four-micron frozen sections were cut, air-dried and fixed in cold acetone (10 min). Indirect immunofluorescence was performed as follows:

(a) incubation of sections with anti-D47 (dilution 1:50 to 1:100, 45 min, 37°C)

(b) washing in phosphate-buffered saline (PBS, pH 7·2, 30 min)

(c) incubation with fluorochrome-conjugated second-layer antiserum (Meloy goat anti-mouse Ig, labelled with fluorescein-isothiocyanate, dilution 1:20, 30 min, 37°C)

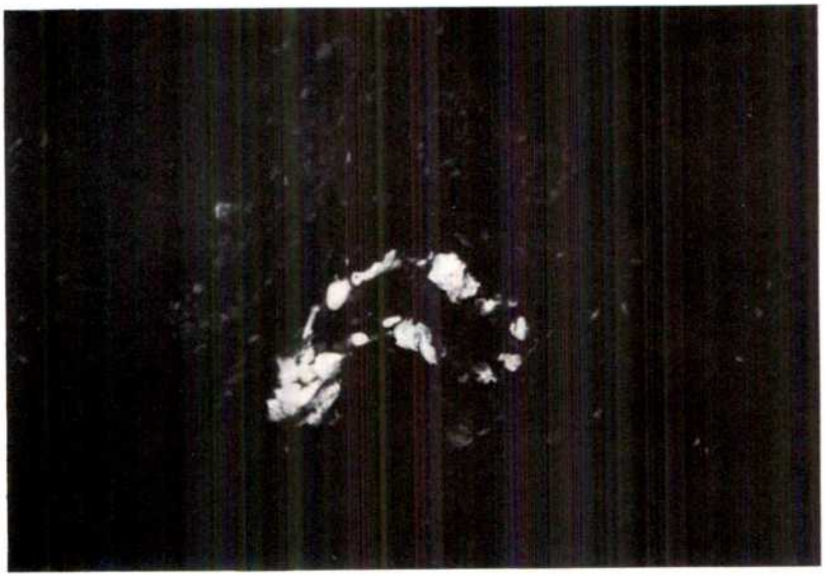
(d) washing as above-mentioned. A counterstaining for nuclei was then performed by flooding the sections with propidium iodide (dilution 1:50, 2 min). After a final washing in PBS, the slides were mounted in modified polyvinyl alcohol medium and examined on a Zeiss fluorescent microscope with epi-illumination.

RESULTS

No labelling was observed on animal tissues, even on mouse foot-pad skin, known to be rich in sweat glands. However, on the monkey skin sections, pilosebaceous follicles but no sweat glands were present.

On human thymus, anti-D47 reacted with a surface antigen of cortical thymocytes.

On normal human facial skin, anti-D47 was found to react with a cytoplasmic antigen of the cells lining the deep portion of ESG (Figs 1 and 2). No labelling was observed on the intradermal or intraepidermal excretory duct (acrosyringium). On closer examination, it could be seen that the non-reacting portion of the gland comprised two layers of cells, whose luminal surface was lined by a faint greenish, homogenous rim. This corresponds to the excretory part of the gland, which comprises a double-cell layer lined by an eosinophilic, homogenous cuticle (part of the sweat gland glomerulus, dermal duct, acrosyringium), as seen by standard histological examination (Fig. 3). On the other hand, apocrine as well as sebaceous glands were not stained by anti-D47.



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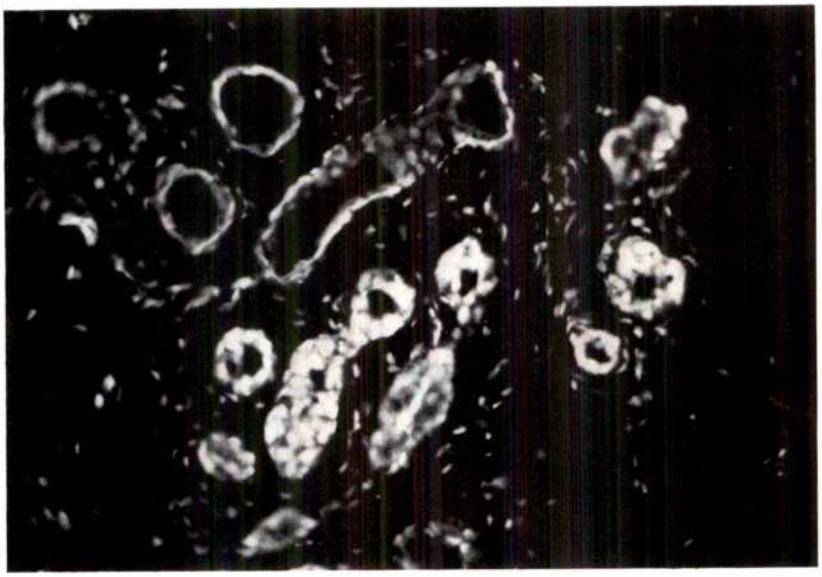

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FIGURES 1 and 2. Cytoplasmic fluorescence of the cells lining the deep portion of human ESG glomerulus. Note absence of labelling of part of the ESG glomerulus and of the intradermal duct (counterstaining of nuclei with propidium iodide, × 250).

On biopsies from vulvar Paget's disease, ESG located in the deep dermis showed the same type of staining as that observed on normal skin. However, no labelling of the malignant intraepidermal (Paget's) cells was observed. No staining was observed in the cases of basal cell epitheliomas.

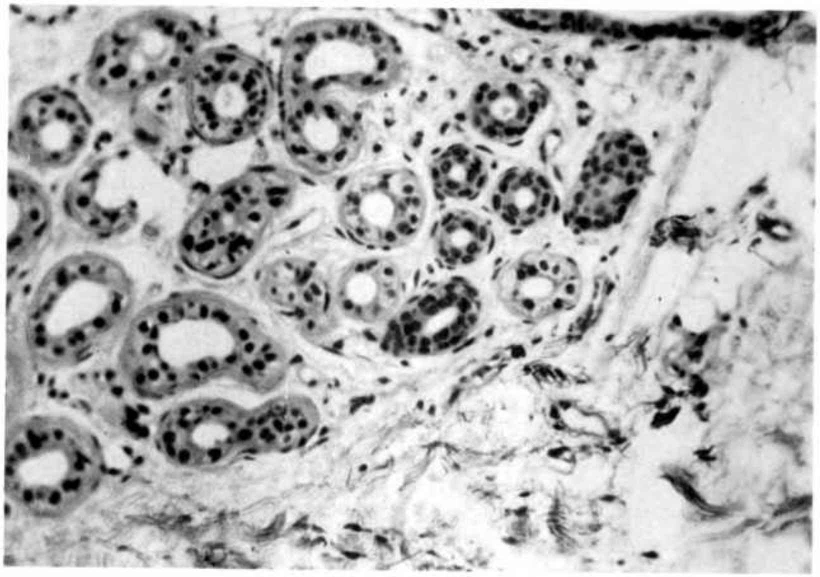

FIGURE 3. Human ESG glomerulus. Note the difference between the secretory and the excretory portion, the latter comprising a basophilic double-cell layer lined by an eosinophilic cuticle (Haematoxylin & eosin, × 250).

DISCUSSION

Anti-D47 is a new monoclonal antibody, prepared against human thymocytes. The surface antigen with which it reacts is a class I antigen of 47,000 molecular weight; it is different from the T6 antigen, as was shown by sequential immunoprecipitation on human thymus gland. It also appears to be human-specific, since anti-D47 did not stain the animal tissues studied.

On human skin, anti-D47 was found to react with a cytoplasmic antigen of the cells lining the deep portion of ESG glomeruli. The whole excretory part of the gland comprising a double-cell layer, the lumen of which is lined by a faint green homogenous cuticle, is not stained. This is strong evidence that the antigen recognized by anti-D47 is expressed by the secretory cells of ESG. Immunoelectronmicroscopic studies have already been undertaken to confirm this statement.

On apocrine and sebaceous glands no labelling was observed. In the case of extramammary (vulvar) Paget's disease, anti-D47 labelled the deep portion of ESG. However, no labelling of intraepidermal Paget's cells was observed. This is in accordance with the view that extramammary Paget's disease arises from an underlying carcinoma of apocrine sweat glands.

Basal cell epitheliomas are in some cases considered to show sweat-gland differentiation (Lever & Schaumburg-Lever, 1975). The cases we studied were of the solid (undifferentiated) type and they did not show any labelling.

Preliminary results of the investigation of eccrine sweat gland tumours are in accordance with the above-mentioned results (unpublished data).

Several enzymatic markers of ESG are already known (e.g. amylophosphorylase, cytochrome-oxidase, succinic dehydrogenase) which allow a distinction between eccrine and apocrine structures. However, these markers are equally present in the excretory and the secretory part of ESG. Anti-D47 appears to be an immunological marker labelling specifically secretory cells of

ESG. Provided this specificity is confirmed by ultrastructural studies, anti-D47 will be a new tool for investigating sweat gland-related pathology, with special reference to the origin of adnexal skin tumours showing glandular differentiation.

On the other hand, the unexpected reactivity of anti-D47 with the secretory cells of ESG is of great interest and remains to be discussed. As mentioned previously, the antigen of the cortical thymocytes with which anti-D47 reacts is a class I antigen of 47,000 molecular weight. However, it does not seem very probable that the same molecule is expressed by the secretory cells of ESG. It is likely that a small antigenic determinant could be shared by these cells and cortical thymocytes. Examples of monoclonal antibodies have recently been reported that react with epitopes common to unrelated cells or molecules (e.g. cytoplasmic filaments) (Thivolet *et al.*, 1983). Monoclonal antibody anti-D47 may become a useful tool for the localization of eccrine sweat gland antigens.

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Human skin battery potentials and their possible role in wound healing

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SUMMARY

Measurements of transcutaneous voltage have been made on seventeen normal volunteers. The results show the presence of 'skin battery' voltages comparable in size to those previously reported for amphibian and mammalian skin. No correlation was found between battery voltage and age or sex in the group studied, but consistent anatomical variations were observed. The possible role of these voltages in the natural wound healing process is discussed.

The ability of several species, for example some amphibians, to regenerate lost body parts is well documented. Recent work has shown the presence of natural direct currents in the vicinity of regenerating amphibian limbs driven by so-called skin batteries (Borgens, Vanable & Jaffe, 1977a; Jaffe & Nuccitelli, 1977). These currents have been measured with the aid of a vibrating probe, a device which enables the measurement of current densities entering or leaving a biological source immersed in an aqueous conducting medium and overcomes the problems of d.c. electrode offset potentials (Jaffe & Nuccitelli, 1974). It has been shown that these currents are essential for normal regeneration and that their reversal will induce degeneration. The source of the currents appears not to be related to motor or sensory nerves as they are also present in denervated limbs. The currents have been shown to come from the epidermis and can be altered by changing the sodium concentration of the medium or by blocking the sodium uptake by the skin with amiloride or methyl ester of lysine (Borgens *et al.*, 1977a). It has also been shown that if currents are artifically introduced into species such as the frog, which are not natural regenerators, then partial regeneration of an amputated limb will be initiated (Borgens *et al.*, 1977b). These regenerated frog limbs contain a cartilage core, nerve fibres and mature thorn-shaped epidermal papillae as would be found in a normal adult frog limb.

It has been reported that children's fingertips will regenerate with excellent cosmetic and functional results if treated conservatively after accidental amputation distal to the distal

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inter-phalangeal joint (i.e. no suturing, skin grafting or other surgical procedures performed) (Illingworth, 1974; Rosenthal, Reiner & Bleicher, 1979). With the aid of a hand-held vibrating probe (Barker, 1981) it has been shown that amputated fingertips in children produce current densities of up to 35 $\mu A/cm^2$ flowing for several days during the regeneration process, similar to those demonstrated in amphibians (Illingworth & Barker, 1980).

Further investigation of the source of these currents in mammalian skin has been performed on the foot pad of the guinea-pig (Barker, Jaffe & Vanable, 1982). This area was chosen because of the absence of sweat glands and hair follicles, both of which are known to affect the electrical properties of the skin (Fowles & Venables, 1970). Measurements showed that as in amphibian skin, a voltage of approximately 40 mV exists across the epidermis with the stratum corneum always negative with respect to the dermis. These batteries have been shown to lie within the epidermis below the stratum corneum but above the basement membrane. In addition it was found that the potential difference across the epidermis in intact skin could drive d.c. currents into epithelial wounds with an amplitude of approximately 1 μA per mm of wound perimeter. With the use of microelectrodes a voltage gradient was shown to exist in the intact skin within 1 mm of the wound edge, reaching as high as 200 mV/mm.

The object of this study was to determine whether human skin possesses similar battery potentials to those already demonstrated in other mammals and amphibians, and whether there is any variation with anatomical site, age or sex.

METHODS

Seventeen normal subjects (eight females, nine males) with an age range of 22–70 years were studied. Skin potentials were recorded between a reference electrode in electrical contact with the dermis and a mobile electrode at 121 predetermined positions on the skin. We have been able

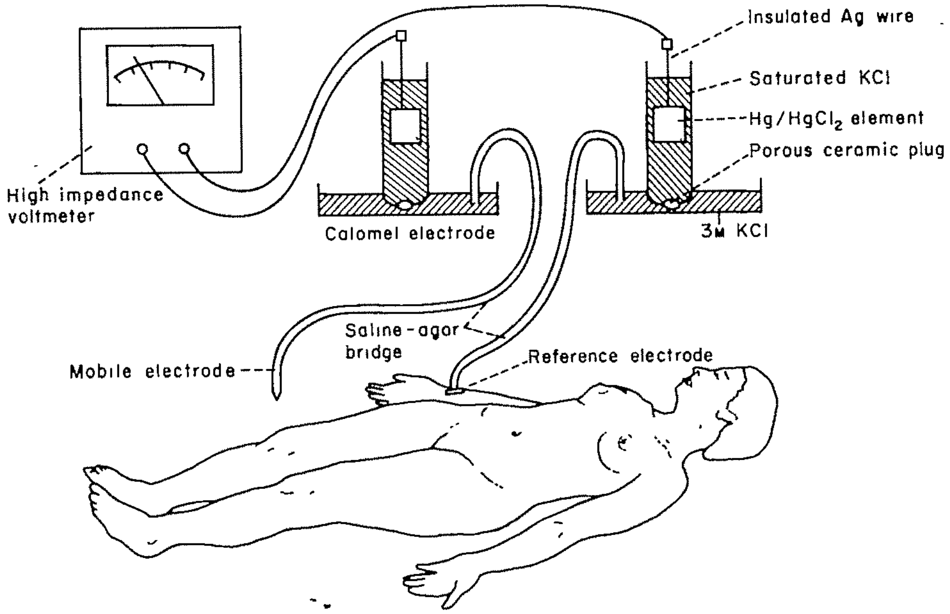
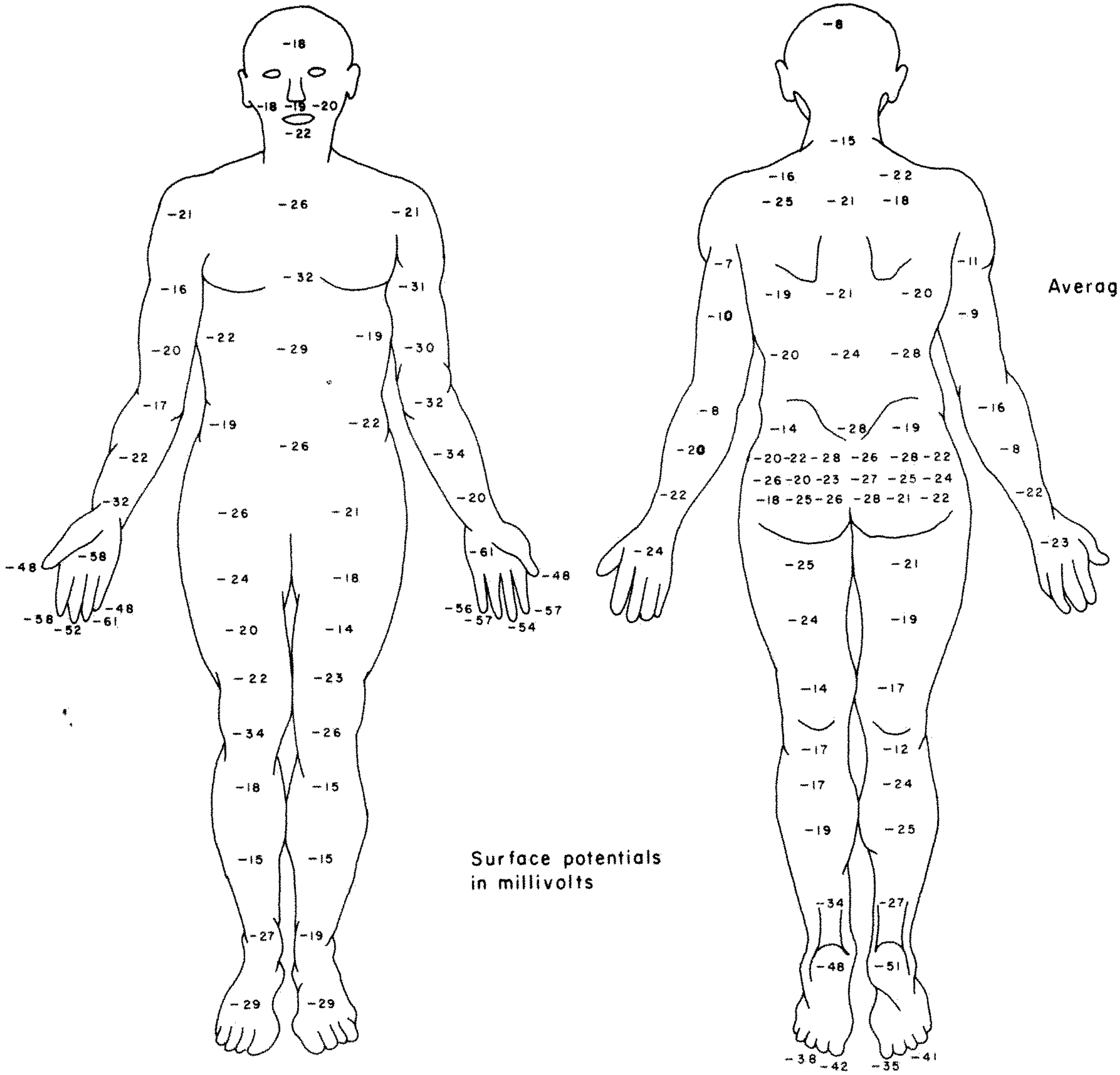


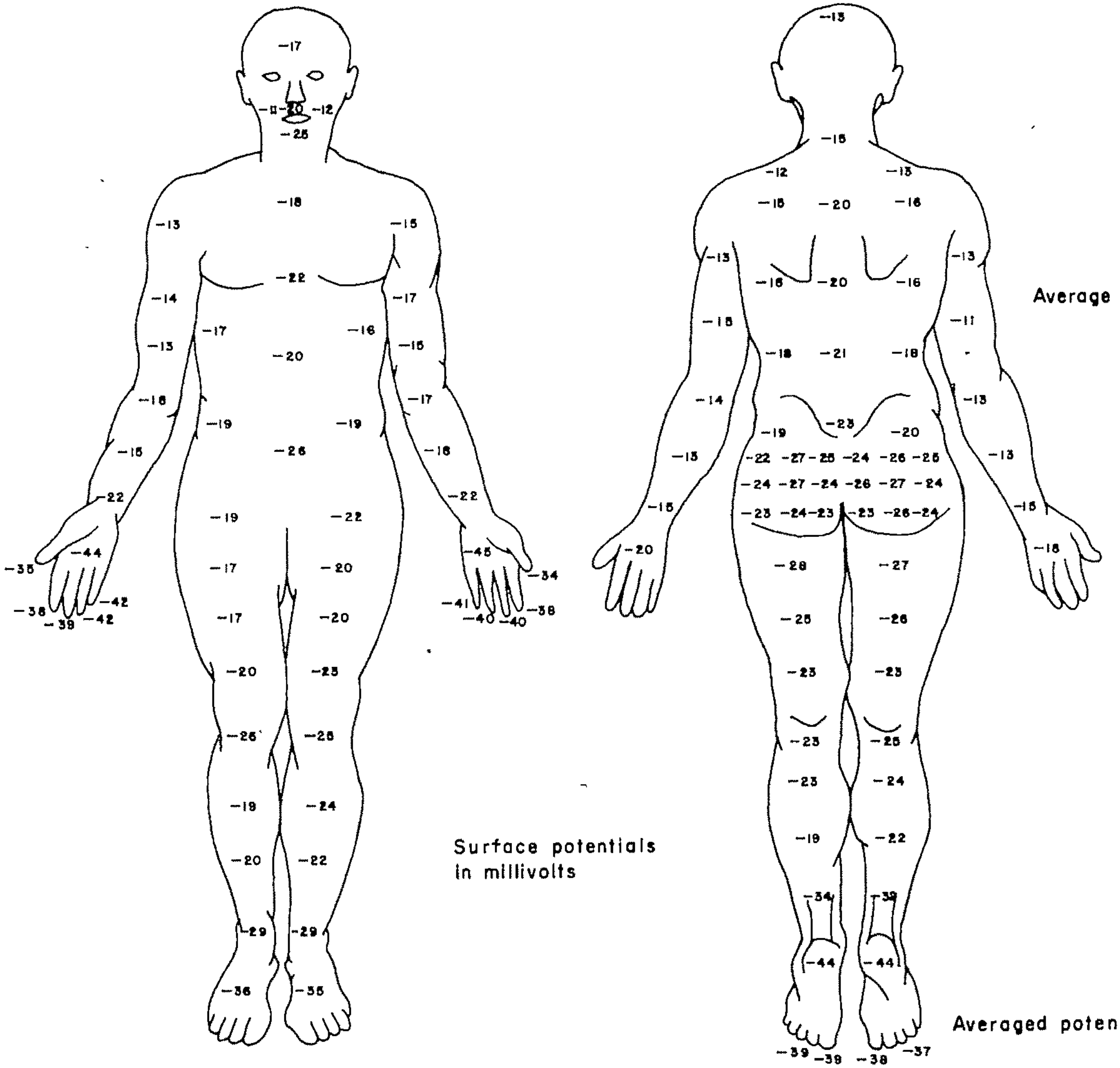
FIGURE 1. Schematic diagram of the recording apparatus.



Average potential and standard deviation

All points	−25·7	12·1
Buttocks	−23·9	3·1
Right hand	−49·7	12·8
Left hand	−49·9	13·2
Feet	−39·1	8·1
Right leg	−21·8	5·6
Left leg	−20·0	5·7
Right arm	−17·3	7·2
Left arm	−21·4	10·3
Back	−20·7	4·2
Front	−23·7	4·1
Head	−17·5	4·9

FIGURE 2. Potentials recorded from a typical subject, a man aged 29.



Average potential and standard deviation

All points	−23·4	8·6
Buttocks	−24·7	1·5
Right hand	−36·9	8·8
Left hand	−36·9	8·1
Feet	−39·0	3·4
Right leg	−23·4	4·5
Left leg	−24·1	4·0
Right arm	−14·7	3·2
Left arm	−16·2	3·5
Back	−17·5	3·1
Front	−18·8	3·5
Head	−16·3	5·4

Averaged potentials for first seventeen normals

FIGURE 3. Average potentials for all individuals at each site.

to show, by experiments on ourselves, that there is less than a 1 mV potential difference between any two dermal sites making contact with the human body by means of measurements made at multiple sites making contact with the dermis. Therefore only one dermal reference connection is required, which allows a mobile surface electrode to measure the transcutaneous potential beneath it without the need for multiple skin punctures. We found that an effective dermal contact could be simply obtained by the use of a Heaf gun on the volar aspect of the forearm, with the reference electrode placed over the puncture site. The integrity of this dermal contact could be checked during the experiment by placing the mobile electrode on another Heaf gun site wound which then recorded a zero potential difference.

The electrodes consisted of cotton wool soaked in physiological 0·9% saline, connected by means of a saline–agar bridge to calomel electrodes immersed in 3 M KCl. These bridges were made by filling plastic tubes, 120 cm long and 3 mm internal diameter with a solution of 1·5 g of agar dissolved in 100 ml of 0·9% saline.

Silver/silver chloride electrodes were not chosen because of the problems of electrode offset potential and because they are sensitive to the concentration of chloride ions. Calomel electrodes have the advantage of a stable electrode offset potential of less than 1 mV, and the saline–agar bridge allows chemical isolation of the electrodes from the patient. A high impedance voltmeter ($10^{12}\Omega$) was chosen to avoid measurement errors due to skin impedance which can be as high as 10 MΩ/cm^2 in areas of ichthyotic epidermis.

The measurements were made with the subject supine in an air-conditioned room. The predetermined electrode sites were chosen to cover most of the skin surface and were consistent in all subjects. The mobile electrode was held with minimum pressure to maintain contact with the stratum corneum via a drop of saline covering an area of approximately 10 mm^2. Fig. 1 shows a schematic diagram of the recording apparatus.

RESULTS

Negative potentials from the stratum corneum with respect to the dermis were recorded consistently at all sites examined and in all individuals. The average potential over all sites and subjects was −23 mV with a standard deviation of 9 mV.

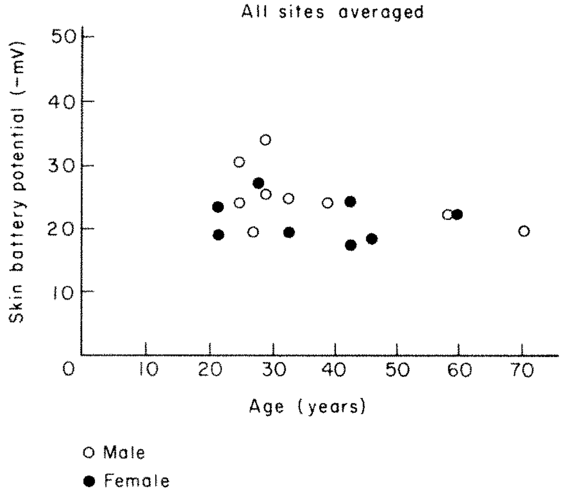


FIGURE 4. Average potentials recorded for all sites related to age and sex of subjects.

Fig. 2 shows a map of the recordings made from a typical subject and Fig. 3 shows the average potentials at each site for all subjects examined. The results showed a consistent anatomical variation, with the battery voltages recorded from the hands and feet significantly higher than elsewhere. Fig. 4 shows the average potentials recorded for all sites related to age and sex of the subjects. For the age range examined there appeared to be no significant correlation between skin battery voltages and age or sex. However, a consistent variation of battery potential with anatomical site was found to exist. Areas such as the hands and feet have relatively high battery potentials whereas the back and upper arms have relatively low potentials. All subjects demonstrated this general variation.

DISCUSSION

The measurements have been made across the skin, that is to say between an electrode in contact with the stratum corneum and an electrode in contact with the dermis. Electrical properties of the skin have been used for many years in psychological studies to assess emotional state. The two main measurements used have been the electrical resistance of the skin, as an indicator of sweat gland behaviour, and surface potential measurements, either between two intact sites or between an intact site and an abraded area (see for example, Christie, 1981). By using a transcutaneous recording system we have obtained results which will combine potentials due to structural characterisitics of the epidermis and due to electrical activity originating from eccrine sweat glands (Wilcott, 1966). Hence a correlation between skin battery voltage and sweat gland distribution might be expected to exist. The higher voltages measured over the palms of the hands and soles of the feet are compatible with this, but the relatively low voltages recorded on the forehead suggest there may also be substantial variation in sweat gland independent 'basal' skin potential levels. A possible explanation for this regional variation may be epithelial thickness. Additionally, it has been shown that there is sharp depression of transcutaneous potentials in hairy regions of mammalian skin probably due to current leakage along hair follicles (Barker *et al.*, 1982). Hence, the anatomical variation of skin battery potentials is a complex combination of several factors and does not appear to be related to dermatone distribution. The apparent lack of correlation between battery potentials and age up to 70 years is surprising in view of the known ageing process of the skin, with diminishing epidermal thickness and decreased sweat production.

The potentials we have measured are of a similar magnitude to those already demonstrated in other mammals and amphibians, and which have been shown to generate large voltage gradients

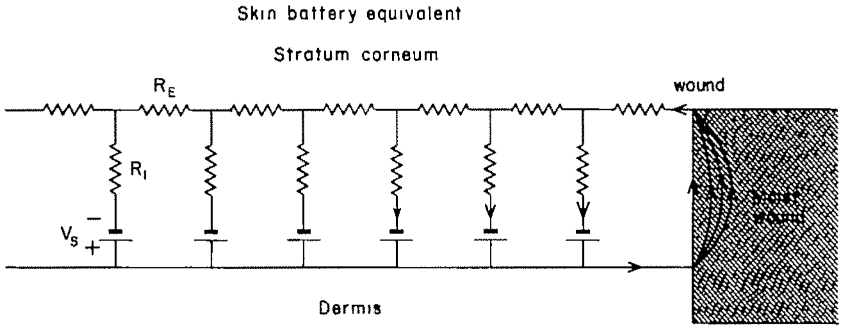


FIGURE 5. Schematic representation of current flow at a wound edge.

at the edges of epithelial wounds (Barker *et al.*, 1982). For practical reasons, it has not been possible to measure voltage gradients in the vicinity of experimental wounds in humans. However, the fingertip current measurements made in children showed that human skin batteries are capable of driving substantial currents into wounds and voltage gradients similar to those measured in the guinea-pig can be expected to exist. These voltage gradients may have a significant effect on cellular function at wound margins. Fig. 5 shows a schematic representation of current flow at a wound edge.

One of the important mechanisms of wound healing is the process of re-epithelialization. Studies using pigs have shown that the cells migrating across a wound come from a strip of intact skin approximately 0·5 mm wide around the wound (Winter, 1964) and it is in this area that substantial voltage gradients occur. The mechanisms by which cells migrate are not understood but one possibility is that they may move under the influence of voltage gradients either passively, for example by electrophoresis, or by active polarization of charged molecules in the cell membranes. Some cells are known to become orientated in voltage gradients as low as 7 mV/mm (Hinkle, McCaig & Robinson, 1981) which is considerably lower than the expected voltage gradients at a wound margin.

The existence of epidermal batteries and the resultant voltage gradients at the edges of intact skin may also explain the phenomenon of epiboly in organ culture whereby epidermal cells migrate around the edge of the culture and subsequently grow back into the dermis (Reaven & Cox, 1965). This cell movement would appear to be along the expected path of current flow.

It is tempting to speculate as to whether skin batteries are connected with acupuncture sites. Whilst there is no evidence of this it can be expected that a needle which penetrates the epidermis will cause local currents to flow and these may have a similar effect to those deliberately applied in electro-acupuncture.

There has been some clinical evidence that electrical stimulation of wounds may encourage wound healing (Wheeler *et al.*, 1971) and indeed this may act by enhancement of the skin batteries. If wound healing is mediated by electrical signals, at least in part, the possibility exists of electrically stimulating wounds to enhance the healing process.

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Altered binding of *Ulex europaeus* I lectin to psoriatic epidermis

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SUMMARY

We have used *Ulex europaeus* I (UEA I) lectin, specific for α-L-fucose-containing glycoconjugates, in fluorescence microscopy to stain cryostat sections of human skin from normal persons and patients with psoriasis and lichen simplex. In normal skin the upper layers of the stratum spinosum and the stratum granulosum were strongly reactive with UEA I, whereas the lower layers of the epidermis did not react. The staining intensity of the upper epidermis was similar to that of the endothelium of dermal blood vessels. Biopsies of the lesional skin of lichen simplex showed an intense UEA I-specific staining throughout the whole epidermis, similar in intensity to that seen in the upper epidermis of normal skin.

In psoriatic lesions positive UEA I-specific fluorescence was seen throughout the whole epidermis, but the fluorescence was more faint and often granular. In uninvolved skin of psoriatic patients the whole epidermis showed a diffuse UEA I-specific fluorescence, differing in this respect from normal skin.

In normal skin UEA I binds to epidermal cells which are at a certain state of differentiation. The results with psoriatic epidermis confirm that both uninvolved and lesional epidermis have a defect in epidermal maturation, as shown by the altered binding of UEA I lectin.

Psoriatic keratinocytes appear to have altered surface properties (Orfanos *et al.*, 1973; Gommans *et al.*, 1979), which could partly explain the disturbances in cell proliferation and differentiation found in the disease (Mahrle & Orfanos, 1977). Changes in labelling with radioactive sugars (Roelfzema *et al.*, 1981) in binding of some lectins (Gommans *et al.*, 1982) and also specific surface glycoprotein alterations (Kariniemi, Lehto & Virtanen, 1983) have been found in epidermal cells in psoriasis.

Lectins have been used to characterize cellular saccharide moieties both *in vivo* and *in vitro* (Lotan & Nicolson, 1979). Studies on rodent epidermis with fluorochrome-coupled lectins have

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revealed that some lectins bind to distinct layers of epidermis (Brabec *et al.*, 1980). Results on human skin have been more conflicting (Holt, Hill Anglin & Nordquist, 1979; Reano *et al.*, 1982a,b) but it appears that psoriatic epidermal cells show alterations at least in binding of some α-fucosyl-specific lectins, such as *Ulex europaeus* agglutinin I (UEA I) (Gommans *et al.*, 1982). In this respect it is interesting that in line with results on rodent epidermis (Brabec *et al.*, 1980) UEA I lectin appears to react selectively with the upper Malpighian and granular cell layers of normal human epidermis in addition to dermal vascular endothelium (Holthöfer *et al.*, 1982). In the present study we have investigated the binding of fluorochrome-coupled UEA I lectin to both lesional and uninvolved epidermis in psoriasis compared with the binding in normal and in the hyperplastic skin of lichen simplex.

METHODS

Tissue samples. Punch biopsies, 2 mm in diameter, were taken at the Department of Dermatology, Helsinki University Central Hospital, from twenty-three patients with clinically and histologically verified psoriasis. Specimens were taken from the psoriatic plaque and from uninvolved skin of the arm. Seventeen of the patients suffered from the usual plaque type of psoriasis, four of the patients had the guttate type of the disease and two patients had psoriatic erythroderma (biopsies from uninvolved skin could not be taken from these patients). Biopsies from the uninvolved skin of the arm were also taken from three patients with psoriasis of Reiter's type and from one patient with the acrodermatitis of Hallopeau type of acral psoriasis. Patients with pustulosis palmoplantaris were not included in this study. The control biopsies from

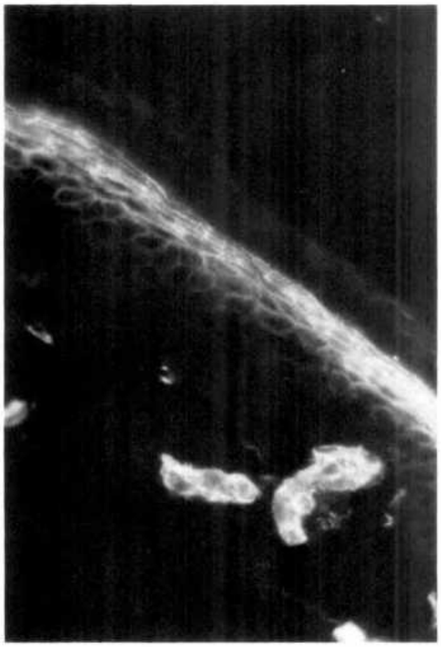

FIGURE 1. In normal skin TRITC-UEA I give a bright staining of the upper layers of the stratum Malpighii and the granular cell layer, in addition to the dermal vessel endothelium, but the basal cells are unstained (× 310).

normal skin were taken in surgical operations from different skin areas from ten patients with no history of psoriasis. Additional control biopsies from hyperplastic skin were taken from six patients with lichen simplex chronicus. The samples were frozen in liquid nitrogen and stored at −70°C until used.

Staining with fluorochrome-coupled lectin. Tetramethylrhodamine isothiocyanate-coupled *Ulex europaeus* I lectin (TRITC-UEA I) was obtained from Vector Laboratories (Burlingame, U.S.A.). Many other commercial conjugates were tested but were unsatisfactory. The specificity of the lectin binding was controlled both by pre-incubation of the lectin conjugate with α-L-fucose as described earlier (Holthöfer *et al.*, 1981) and by treating the sections with L-fucosidase (Boehringer, 4 U/ml in acetate buffer, pH 6·0, at 37°C for 30 min). Both treatments inhibited the staining. The lectin conjugate was applied to the sections at a concentration of 100 μg/ml at room temperature for 30 min. Thereafter the specimens were washed thoroughly and embedded in buffered nicotamide. The specimens were examined in a Zeiss Universal microscope equipped with an epi-illuminator III RS and filters for TRITC-fluorescence.

RESULTS

In normal skin the keratinocytes in the upper spinous cell layers and in the granular cell layers were strongly positive for fluorochrome-coupled UEA I lectin whereas the cells in the lower epidermal layers did not show any binding (Fig. 1). The keratinized layer was also negative for UEA I. The pattern of fluorescence was linear, locating at the peripheries of the keratinocytes. The staining intensity was comparable to that of the endothelium of dermal blood vessels.

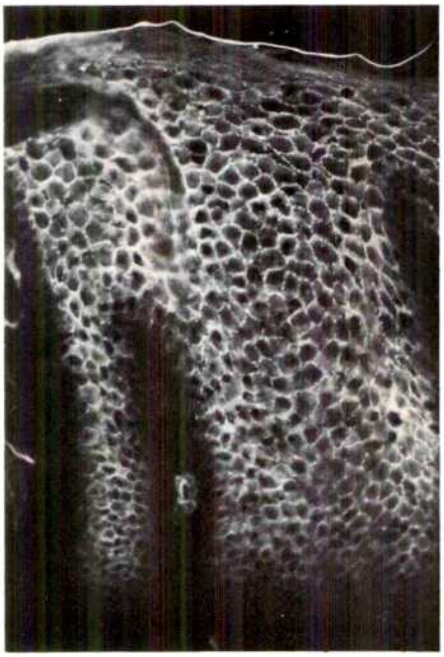

FIGURE 2. In a psoriatic lesion a fine granular TRITC-UEA I-specific staining along cell borders is seen throughout the whole thickened epidermis (× 310).

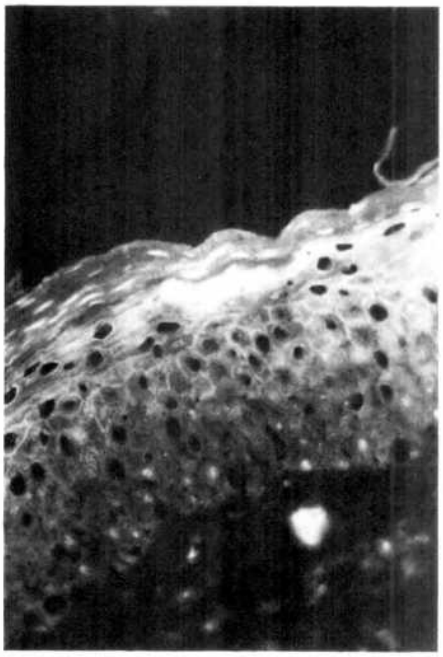

FIGURE 3. In uninvolved skin of psoriatic patients a diffuse TRITC-UEA-I-specific staining is seen throughout the whole epidermis including the basal cells. Note the bright staining of dermal vessel endothelium (× 310).

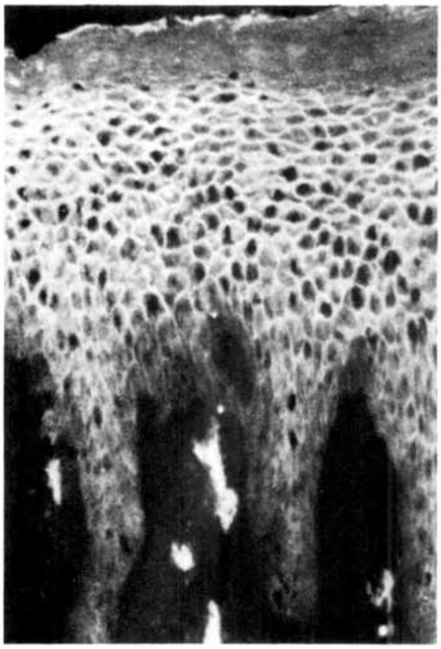

FIGURE 4. In the hyperplastic epidermis of lichen simplex all the keratinocytes express bright TRITC-UEA I-specific fluorescence. Dermal blood vessel endothelia are also brightly stained (× 310).

In skin biopsies derived from psoriatic lesions more faint UEA I-positive staining was observed throughout the whole thickened epidermis (Fig. 2). The parakeratotic keratin layer was only faintly stained with TRITC-UEA I. The staining of psoriatic epidermis was not so intensive as that of upper layers of normal skin and the fluorescence pattern was often granular. The same staining pattern was found in all seventeen cases with plaque type psoriasis, in all four cases of guttate psoriasis and in both cases of psoriatic erythroderma.

In biopsies of uninvolved skin from psoriatic patients a diffuse UEA I-positive fluorescence was also seen throughout the whole epidermis including also the basal cell layer (Fig. 3). Such a staining pattern of uninvolved skin was found in all cases of plaque psoriasis and in three cases of guttate psoriasis. In one patient with guttate psoriasis the uninvolved skin showed an intermediate type of fluorescence: an obscure fluorescence throughout the whole epidermis with a bright area at the granular layer. A diffuse 'psoriatic' type of TRITC-UEA I binding to the uninvolved skin was seen also in three patients with psoriasis resembling Reiter's disease and in one patient with acrodermatitis Hallopeau.

In order to find out whether the faint, granular staining pattern with UEA I lectin found in hyperplastic psoriatic skin is typical also for non-specific epidermal hyperplasia, biopsies from lichen simplex chronicus were also studied. In lichen simplex all the keratinocytes in the thickened epidermis expressed a bright linear staining surrounding each keratinocyte as in the upper epidermal layers in normal skin (Fig. 4). TRITC-UEA I-specific staining pattern in uninvolved skin of patients with lichen simplex was comparable to that seen in normal skin.

DISCUSSION

In the present study UEA I lectin was found to bind to keratinocytes in the upper Malpighian and granular cell layers in normal human epidermis. In psoriasis, the keratinocytes in both diseased and uninvolved epidermis express an altered pattern of UEA-I binding throughout the whole epidermis.

Previous studies using intact skin have indicated that differentiated keratinocytes in the upper layers of rat (Brabec *et al.*, 1980) and human (Holthöfer *et al.*, 1982) epidermis bind UEA I lectin, thus expressing certain α-L-fucosyl-containing glycoconjugates on the cell surface. Thus binding of UEA I to keratinocytes seems to reflect a certain stage in epidermal cell differentiation (Brabec *et al.*, 1980). Fucosyl residues, recognized by UEA I lectin, and other terminal saccharides are found in addition to the cell surface only at the Golgi apparatus of cells (Virtanen, Ekblom & Laurila, 1980). Thus, staining obtained with this lectin is mainly due to cell surface-associated glycoconjugates.

Enzymatically isolated keratinocytes from normal skin and from both diseased and uninvolved psoriatic epidermis also appear to bind UEA I lectin (Gommans *et al.*, 1982). However, contradictory results on binding of UEA I to epidermal cells have also been reported: Reano *et al.*, (1982a,b) did not find any binding of UEA I lectin to either normal or psoriatic epidermis. Further, isolated keratinocytes and melanocytes from guinea-pig skin were found to be negative for UEA I in primary mixed epidermal cell cultures (Schuler, Romani & Fritsch, 1982). The differences in the results on binding of UEA I lectin to mammalian epidermis presumably are due to inactivity of certain commercial lectin preparations as repeatedly found in our laboratory.

Using enzymatically isolated normal and psoriatic keratinocytes, Gommans *et al.* (1982) found an increased affinity of UEA I lectin to the surface of psoriatic keratinocytes although the total quantity of bound lectin was similar for normal and psoriatic epidermis. The same phenomenon was found to a lesser extent also in keratinocytes derived from uninvolved psoriatic skin. Using fluorescence microscopy at the tissue level it is difficult to evaluate the amount of UEA I lectin bound to individual cells. The number of UEA I-binding cells appears to be clearly increased not only in psoriatic lesions but also in the uninvolved skin of psoriatic patients. However, the intensity of UEA I-specific fluorescence in psoriatic keratinocytes is lower than in normal epidermis or in dermal blood vessel endothelium and the pattern is often granular. The bright linear fluorescence typical of the upper layers of normal epidermis is not found in psoriatic lesions, suggesting a lack of mature keratinocytes in the diseased epidermis.

Interestingly, the distribution of UEA I binding to uninvolved psoriatic epidermis, including staining of basal cells, differs from that of normal skin. This is in accordance with previous results of Gommans *et al.* (1982), who using UEA I and other lectins, found changes in the composition of surface sugars of uninvolved psoriatic keratinocytes compared with normal skin. We have recently shown that cultured human keratinocytes derived from uninvolved skin of psoriatic patients express an altered pattern of sialylated surface glycoproteins compared with the surface of cultured normal keratinocytes (Kariniemi *et al.*, 1983). Both these findings suggest an altered composition in surface glycoconjugates of keratinocytes in psoriasis, expressed also in uninvolved skin. In addition, the psoriatic keratinocytes from both the lesion and uninvolved area show an abnormal proliferation after grafting to nude mice, suggesting structural alterations also in uninvolved keratinocytes (Krueger, Chambers & Shelby, 1981).

According to our study, binding of UEA I to the keratinocytes in psoriatic lesions differs qualitatively from that of non-specific skin hyperplasia (lichen simplex). However, we do not yet know whether it is specific for psoriasis or is found also in other proliferative skin diseases. The skin biopsies in this study were taken during the active phase of psoriasis. It would be interesting to see whether the altered UEA I-binding pattern of uninvolved skin is retained during remission or is found even before the onset of the disease, in which case it would be useful for the diagnosis of psoriasis.

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Surface glycoproteins of cultured human keratinocytes from normal and uninvolved psoriatic epidermis

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SUMMARY

Surface glycoproteins of cultured human keratinocytes from normal skin and uninvolved psoriatic epidermis, isolated by the suction blister method, were studied by two different methods. Cells were cultured on collagen-coated culture dishes and showed a fibrillar keratin-specific staining by immunofluorescence. Surface labelling experiments using the neuraminidase/galactose oxidase/sodium borohydride method (which labels the penultimate galactose moieties of glycoproteins) revealed one major glycoprotein with M_r 53 kD (kilodaltons) both in normal keratinocytes and in keratinocytes from uninvolved psoriatic skin. The periodate/sodium borohydride method (which labels the terminal sialic acids in glycoproteins) by contrast revealed three major glycoproteins, with M_r 53 kD to 63 kD, in normal keratinocytes but only a single major glycoprotein, with M_r 53 kD, in keratinocytes from uninvolved psoriatic skin.

Treatment of cultured keratinocytes with etretinate appeared to restore the normal pattern of surface glycoproteins in uninvolved psoriatic keratinocytes.

Recent studies suggest that cell surface properties of keratinocytes may be altered in psoriasis (Mahrle & Orfanos, 1977). Both electron microscopic and functional alterations have been described in psoriatic keratinocytes (Orfanos *et al.*, 1973; Gommans *et al.*, 1979a, b). The composition of cell surface glycoconjugates in normal and in lesional and uninvolved psoriatic human keratinocytes has been studied using specific sugar-binding lectins (Gommans *et al.*, 1982) and metabolic labelling using radioactive sugars (Roelfzema *et al.*, 1981; Roelfzema & van Erp, 1983). These studies have suggested an alteration in cell surface glycoconjugates both in lesional and uninvolved psoriatic keratinocytes.

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Aromatic retinoids have successfully been used in the treatment of psoriasis as well as in other skin diseases with disturbed keratinization and increased epidermal cell proliferation (Fritsch, 1981). Vitamin A and its derivatives have various modulatory effects on cell differentiation and glycoconjugate biosynthesis (Lotan, 1980). They also can cause distinct alterations in cell surface glycoproteins in different cell lines (Lewis, *et al.*, 1978; Jetten *et al.*, 1979).

Using radioactive surface labelling techniques specific for galactose- and sialic acid-containing cell surface glycoproteins we have determined the cell surface glycoprotein pattern of cultured human suction blister keratinocytes (Kariniemi *et al.*, 1982) derived from both normal skin and the uninvolved skin of psoriatic patients. Further, we have investigated the effect of an aromatic retinoid, etretinate, on the expression of surface glycoproteins of both types of cultured human epidermal cells.

METHODS

Preparation of keratinocyte cultures

The skin donors were six healthy volunteers and six patients with psoriasis from the Department of Dermatology, Helsinki University Central Hospital. Four patients with psoriasis were treated with dithranol and two of them had no therapy during the experiment. The suction blisters were generated on the healthy abdominal skin of each volunteer, 30–36 blisters of 5 mm in diameter to each test person. The roofs of the blisters were cut off and trypsinized (Kariniemi *et al.*, 1982) and the dispersed keratinocytes were suspended in MCDB-151 medium (Peehl & Ham, 1980) supplemented with 10% fetal bovine serum, hydrocortisone (10 μg/ml, Upjohn), penicillin and streptomycin. The cells were plated into collagen-coated dishes and incubated at 37°C in water-saturated air containing 5% CO_2. Three dishes (6 cm in diameter) were prepared from each test subject.

In a few days keratinocytes proliferated to small cell colonies on the bottom of the dishes and etretinate (Hoffmann La-Roche Ltd, Basel, Switzerland) in a final concentration of 10^{-6} M dissolved in ethanol was added to 1-week-old cultures. The control cells were grown either in the presence of ethanol or in the plain medium. The retinoid-containing and control media were changed every second day.

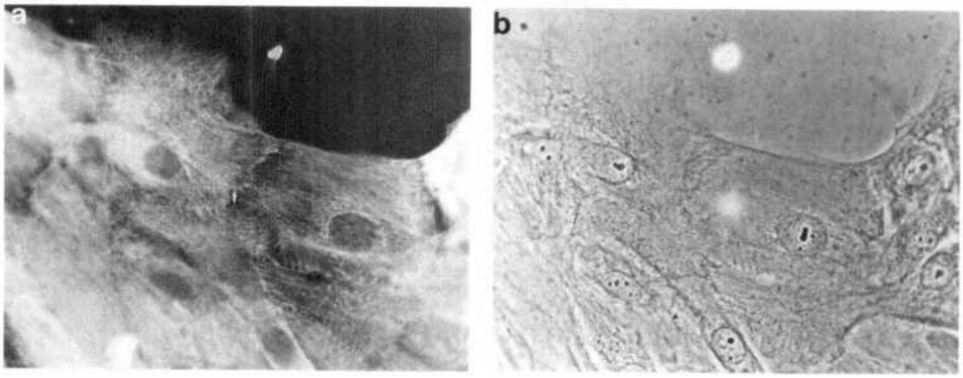

FIGURE 1. (a) Indirect immunofluorescence microscopy with rabbit anti-keratin antibodies shows a fibrillar staining in all cells in keratinocyte cultures. (b) Phase contrast (× 300).

Metabolic and surface labelling and electrophoretic analysis

For surface labelling experiments, the neuraminidase/galactose oxidase/sodium borohydride method of Gahmberg & Hakomori (1973) was used. Briefly, after exposure to retinoid for 2 weeks, the keratinocytes (ca. 5×10^5 cells) from three normal and three psoriatic cell cultures were treated with neuraminidase (12·5 mU ml^{-1}, 12·5 μl; Kabi, Stockholm, Sweden) and galactose oxidase (5 U ml^{-1}; 50 μl; Sigma, St Louis, Missouri) in NaCl-P buffer (1 ml; 140 mM NaCl, 10 mM sodium phosphate, pH 7·4), supplemented with 2 mM $CaCl_2$ at 37 °C for 30 min. After washing twice in NaCl-P buffer, the cells were treated with NaB_3H_4 (2·5 mCi ml^{-1}; 50 μl; 9·8 Ci/mmol, Radiochemical Centre, Amersham, England) in NaCl-P buffer at 23 °C for 30 min. Thereafter the cells were thoroughly washed in NaCl-P buffer. For the metaperiodate/NaB_3H_4 labelling technique (Gahmberg & Andersson, 1977) the cells derived from three other normal and psoriatic persons were first treated with ice-cold 2 mM sodium metaperiodate in NaCl-P buffer for 10 min, washed, scraped into suspension, reduced by NaB_3H_4 as above and washed thoroughly in NaCl-P buffer. After labelling, the cells were dissolved in electrophoresis sample buffer. Equal amounts of radioactivity were loaded for electrophoresis. Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate was performed according to Laemmli (1970) using 6·5% slab gels. After the electrophoresis the gels were first stained for protein with Coomassie blue and were then processed for fluorography according to Bonner & Laskey (1974).

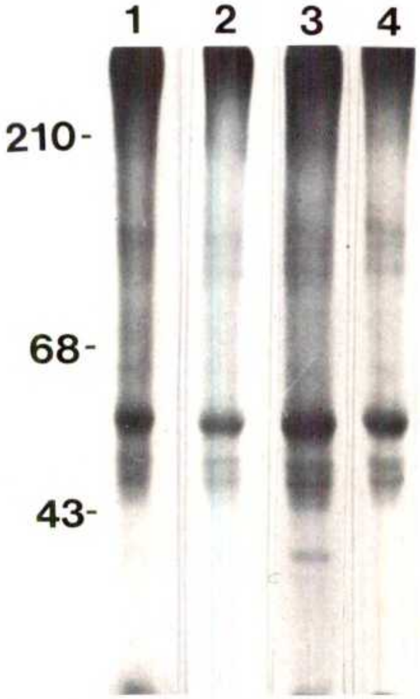

FIGURE 2. Surface glycoprotein profile of normal keratinocytes (lane 1) and keratinocytes derived from uninvolved skin of psoriatic patients (lane 2) using the neuraminidase/galactose oxidase/sodium borohydride method. Exposure of both types of keratinocytes to etretinate (lane 3, normal keratinocytes; lane 4, keratinocytes from uninvolved psoriatic skin) shows a similar glycoprotein profile.

Indirect immunofluorescence microscopy (IIF)

For IIF the cells were cultured on collagen-coated glass coverslips and fixed in −20°C methanol for 10 min. Thereafter the specimens were exposed to rabbit anti-keratin antibodies as described earlier (Kariniemi *et al.*, 1982; Virtanen *et al.*, 1981a,b) followed by fluorescein isothiocyanate (FITC)-coupled goat anti-rabbit IgG antiserum (Cappel Laboratories, Cochraneville, PA). The specimens were examined in a Zeiss Universal microscope, equipped with an epi-illuminator III RS and filters for FITC-fluorescence.

RESULTS

Keratinocytes isolated from the suction blister roofs of both normal skin and uninvolved psoriatic skin rapidly adhered to the collagen-coated culture dishes and formed homogenous cell sheets within 2–3 weeks. In IIF all the cells in such cultures showed a fibrillar keratin organization (Fig. 1a,b), indicating lack of contamination by other cells, like fibroblasts (Kariniemi *et al.*, 1982). Addition of retinoid did not cause morphological changes in cultured keratinocytes.

In surface labelling experiments of normal keratinocytes from three persons a major glycoprotein with M_r 53 kD was seen using the neuraminidase/galactose oxidase/sodium borohydride method (Fig. 2, lane 1), whereas the periodate/sodium borohydride method

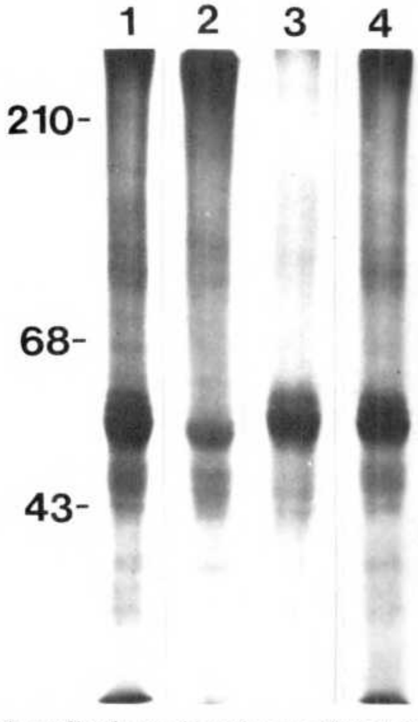

FIGURE 3. Surface glycoprotein profile of normal keratinocytes (lane 1) and keratinocytes derived from uninvolved psoriatic skin (lane 2) using the sodium periodate/sodium borohydride method. Exposure of the cells to etretinate had no influence on the glycoprotein profile of normal keratinocytes with this labelling method (lane 3) whereas the keratinocytes from uninvolved psoriatic skin now show a glycoprotein profile comparable to that of normal keratinocytes (lane 4, compared with lane 1).

revealed a triplet of major polypeptides with M_r 53 kD to 63 kD (Fig. 3, lane 1) in normal keratinocytes derived from three other volunteers. On the other hand, keratinocytes from the uninvolved skin of six psoriatic patients had a single major surface glycoprotein with M_r 53 kD with both labelling methods (Fig. 2, lane 2; Fig. 3, lane 2). No change in the surface glycoprotein profile was seen with either labelling methods when normal keratinocytes were exposed to the aromatic retinoid for 14 days (Fig. 2, lane 3; Fig. 3, lane 3). However, when keratinocytes derived from psoriatic patients were exposed to the retinoid for 14 days, surface labelling with the periodate method showed a glycoprotein profile comparable to that of normal keratinocytes (Fig. 3, lane 4), whereas no change was seen in the surface glycoprotein profile after surface labelling with the galactose oxidase method (Fig. 2, lane 4).

DISCUSSION

Radioactive surface labelling methods require living homogenous cell populations. Using suction blister roof epidermis we have prepared pure human epidermal cell cultures, in which all the cells express fibrillar keratin (Kariniemi *et al.*, 1982). In the present study we have analysed surface glycoprotein profiles of cultured keratinocytes from normal skin and the uninvolved skin of psoriatic patients using sugar-specific surface labelling methods. Psoriatic lesions cannot be properly separated with the suction blister method used.

Only one major glycoprotein with M_r 53 kD was found in both normal and uninvolved psoriatic keratinocytes using the neuraminidase/galactose oxidase/sodium borohydride method. The surface glycoprotein pattern thus differs from most other cells which usually express several polypeptides with M_r between 40 and 220 kilodaltons (cf. Gahmberg & Hakomori, 1976). Recently, the pattern of surface proteins of cultured normal human and rodent keratinocytes has been analysed using lactoperoxidase-catalysed iodination (Brysk & Snider, 1982a,b). The SDS-PAGE of human cells revealed most prominent labelled polypeptides in the low molecular weight region, between 46 kD and 68 kD (Brysk & Snider, 1982a). In undifferentiated rodent keratinocytes, the 79 kD polypeptide was also discernible, but disappeared in differentiated cells (Brysk & Snider, 1982b). This method, however, also labelled collagen, which is required for the attachment and growth of human keratinocytes *in vitro*. On the other hand, it appears that the lactoperoxidase method does not label efficiently all surface glycoproteins, apparently due to a steric hindrance to the probe (Lehto, 1983).

A distinct difference between normal and uninvolved psoriatic keratinocytes was found in the pattern of sialylated surface glycoproteins using the periodate/sodium borohydride labelling method. In normal keratinocytes, three major sialic acid-containing glycoproteins with M_r between 53 kD and 63 kD were found, whereas keratinocytes derived from uninvolved skin of psoriatic patients expressed only one sialylated glycoprotein with M_r 53 kD. This finding suggests that the surface glycoprotein composition of psoriatic keratinocytes in uninvolved skin, where the blisters were generated and the epidermal cells obtained, is different from that of normal keratinocytes. This is in line with the recent results of Gommans and his co-workers (1982), who found a change in Con A binding to both lesional and uninvolved psoriatic keratinocytes. Further, the composition and turnover of cell surface-associated fucose-containing glycoproteins is altered in both kinds of psoriatic epidermal cells (Roelfzema *et al.*, 1981; Roelfzema & van Erp, 1983). It has been shown that the psoriatic keratinocytes, both uninvolved and diseased, proliferate abnormally after grafting to nude mice (Krueger, Chambers & Shelby, 1981). The cell surface membrane is known to be involved in many cell functions during adhesion, growth and differentiation (Mahrle & Orfanos, 1977; Griffin &

Pasternak, 1982). Hence the altered pattern of cell surface glycoproteins could lead to disturbances in cell differentiation and proliferation found in psoriasis.

Interestingly, the abnormal composition of sialylated glycoproteins of uninvolved psoriatic keratinocytes could be 'normalized' with an aromatic retinoid, which affects the proliferation and differentiation of keratinocytes in psoriasis *in vivo* (Fritsch, 1981). Using various cell lines retinoids are shown to cause a change both in the expression of a high molecular weight pericellular matrix glycoprotein, fibronectin, and also in the profile and glycosylation of surface membrane glycoproteins (Lotan, 1980; Sasak *et al.*, 1980).

In summary, our results indicate that keratinocytes derived from uninvolved skin of psoriatic patients differ in their surface glycoprotein pattern from normal human keratinocytes. Etretinate, used successfully in clinical control of psoriasis, appeared to restore the normal pattern of surface glycoproteins in uninvolved psoriatic keratinocytes.

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Studies on the phagocytic activity of the granulocytes in psoriasis and palmoplantar pustulosis

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SUMMARY

A kinetic method was used to determine the rates of phagocytosis of IgG-coated and C3b-IgG-coated latex particles by neutrophil granulocytes from twenty patients with psoriasis and twenty with palmoplantar pustulosis (PPP). Patients with psoriasis showed a highly increased rate of uptake of IgG-coated particles. An increased rate was also observed in patients with PPP. These results indicate enhanced Fc-receptor function. In healthy controls the uptake rate of C3b-IgG-coated particles was at least 30% higher than that of particles coated with IgG alone. In nearly half of the patients in the psoriasis and PPP groups the uptake rate failed to increase in the normal way, indicating that C3b dysfunction is common in both psoriasis and PPP. In neither group was there any relationship between the rate of phagocytosis and the clinical severity of the disease.

Several reports on abnormal granulocytes in psoriasis have been published. The main interest has been devoted to the chemotactic activity. The results have been divergent, possibly due to differences in methods but most findings point to changes in granulocyte function in psoriasis. The phagocytic capacity in psoriasis has been less fully studied and the results have been less conclusive.

Palmoplantar pustulosis (PPP) is a disorder considered to be related to psoriasis, but the leukocytes in PPP have not been investigated to any great extent. In the pustules of PPP, neutrophil granulocytes are the predominant cells, although in the earliest stage of the disorder they are preceded by mononuclear cells. A similar time schedule is also observed in the microabscess formation in psoriasis. In view of the possible association between the two disorders, we have studied granulocyte function in psoriasis and PPP. The results indicate that both the Fc- and C3b-receptor functions are altered in many patients with psoriasis or PPP.

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METHODS

Psoriasis patients. This group consisted of eight women and twelve men aged 18–61 years (mean 38 years) and with a minimum duration of psoriasis of 6 months (mean 16 years). No patients with guttate (post-streptococcal or post-pharyngitic) psoriasis were included.

At the time of blood sampling and clinical examination, all patients had skin lesions, which were of the moderately infiltrated, scaling, nummular and/or plaque type. The areas involved varied. Five had mild and stable psoriasis with lesions only on the elbows, knees and scalp, with less than 10% of the skin affected; three patients with flaring, labile and almost generalized psoriasis had more than 75% involvement, with pronounced erythema and scaling. Two of these patients were erythrodermic and one had pustular psoriasis. The remaining twelve patients had 20–50% involvement. Most of them had experienced a slow, gradual worsening of their lesions during the previous months. Three patients – two with severe psoriasis, one with moderate and one with mild – had joint symptoms without serological signs of rheumatoid arthritis (RA).

The following treatments were used: etretinate 25 mg daily plus methotrexate 7·5 mg weekly (one patient); etretinate 25 mg daily (two patients); intermittent PUVA (three patients); UV-B irradiation (one patient); dithranol (two patients); topical corticosteroids (one patient). The only treatment in the other patients was petrolatum topically.

Palmoplantar pustulosis (PPP) patients. This group consisted of fifteen women and five men aged 17–72 years (mean 53 years).

The mean duration of the disease was 8 years and all patients had had their lesions for more than 6 months.

The diagnosis was based on the typical clinical picture with recurrent yellowish, sterile pustules on the volar aspect of the hands, on the soles and sometimes on the sides of the heels. Occasionally vesicles – which later became pustular – were present. Redness, scales, and brown, dry pustules were also seen in many cases. Eczema and mycotic infection had been ruled out. None of the patients were free from lesions at the time of blood sampling. The activity at the time of the examination was evaluated in two ways: first, by counting the fresh pustules and vesicles as well as the involuting pustules and by classifying the erythema and scaling into grades of 1–4 and estimating the area of the affected surface; second, based on the clinical impression the patients were divided into three groups of severity – mild, moderate and severe. Six patients were considered to have mild, ten moderate and four severe PPP. Those with a mild disorder were found to have 1–30 fresh pustules, those with moderate 30–100 and those with severe 125–700 pustules.

Six of the PPP patients also had minor but typical psoriatic lesions, mainly on the extremities and in no case involving more than 5% of the skin surface area. One patient with severe PPP had joint pains in the hips and the thoracic and sacral spine with no serological signs of RA. One patient had mild diabetes mellitus and one had a tendency to malabsorption after partial resection of the stomach. None of the patients had any treatment, topically or systemically for their skin disease.

Control subjects. Donors of normal blood leukocytes were either apparently healthy laboratory workers or blood donors at the Blood Centre of the University Hospital, Uppsala.

Preparation of granulocytes

Granulocytes were prepared from heparinized blood as described in detail previously (Hällgren & Stålenheim, 1976). The average purity of the polymorphonuclear neutrophils (PMNs) was 90 ± 5% (s.d.). White blood cell (WBC) counts were performed in all patients.

Protein coating of latex particles

Polyvinyltoluene latex particles with a diameter of 2·03 μm and a volume of $4{\cdot}38 \times 10^{-15}$ l were obtained from Coulter Electronics Ltd, Dunstable, Bedfordshire, England. The particles were coated with human IgG (AB Kabi, Stockholm, Sweden) as previously described (Hällgren, Jansson & Venge, 1977) and these are designated IgG-coated particles. To achieve a complement opsonization, washed IgG-coated particles were incubated at 37°C for 10 min in 20% freshly pooled normal serum diluted in Ringerdex® (Pharmacia, Uppsala). The serum was washed away by two subsequent washes in Ringerdex® immediately before the particles were used in the phagocytosis assay. Such particles are designated complement-IgG-coated particles and the main complement component bound to the particles is C3b (Håkansson, Hällgren & Venge, 1980).

Phagocytosis assay

The assay for measuring the initial rate of phagocytosis has been described in detail previously (Hällgren *et al.*, 1977). Briefly, 2×10^9 isolated PMNs in Ringerdex® containing glucose (6·94 nmol/l) were mixed with 20×10^9 IgG- or complement-IgG-coated latex particles, likewise in Ringerdex®–glucose solution in a final volume of 1 ml, in a siliconized glass tube. Incubation took place at 37°C under constant magnetic stirring (1600 r.p.m.). Aliquots of 100 μl were removed from the mixture every minute for 6 min and non-cell-associated particles were counted in an electronic counter (Trombocounter, Coulter Electronics Ltd). The initial rate of disappearance of the latex particles per min was taken as a measure of the initial rate of phagocytosis.

Statistics. Student's *t*-test was used for statistical calculations.

RESULTS

Phagocytosis of IgG-coated particles

Fig. 1 shows the rate of phagocytosis by PMNs in buffer medium. The granulocytes of the psoriasis group ingested the particles at a significantly higher rate than the granulocytes of the controls. The mean rates (± s.d.) in these two groups were 0·81 ± 0·21 and 0·59 ± 0·15 min^{-1} respectively ($P < 0{\cdot}0001$). In the whole group of PPP patients the mean rate was 0·66 ± 0·19 min^{-1}, which was only slightly increased compared with that in the control group ($P < 0{\cdot}05$). However, the fourteen patients with pure PPP had a mean phagocytic rate of 0·77 ± 0·16 min^{-1} ($P < 0{\cdot}01$ versus controls), which did not differ significantly from that in the psoriasis group. On the other hand, the six patients with PPP combined with very mild psoriasis had a mean value of 0·55 ± 0·16 min^{-1}, which thus did not differ from the control value.

Phagocytosis of complement-IgG-coated particles

Fig. 2 shows the rate of phagocytosis of the serum-opsonized particles. Neither the patients with psoriasis nor those with PPP differed significantly in their mean value from the controls. The

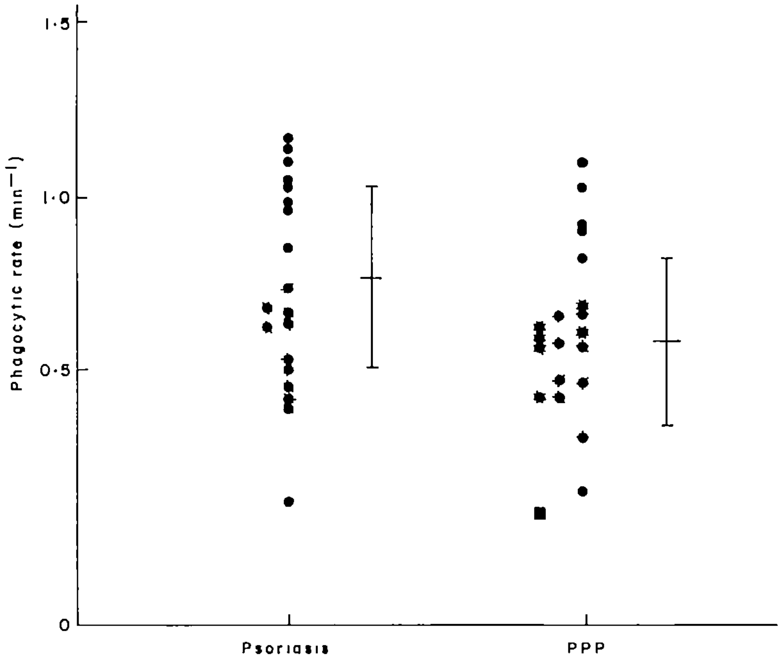

FIGURE 1. The phagocytosis of IgG-coated particles in buffer medium by PMNs isolated from patients with psoriasis (left) and palmoplantar pustulosis, PPP (right). PPP patients with mild psoriasis ■. The phagocytosis of such particles by normal PMNs is indicated by the shaded areas (95% confidence intervals).

mean rate in the psoriasis group was 1·04 ± 0·33, in the PPP group 0·84 ± 0·29 and in the control group 0·94 ± 0·29 min^{-1}. The differences in rate between the psoriasis and PPP patients was weakly significant ($P < 0·05$).

Relation between the rates of phagocytosis of IgG-coated and complement-IgG-coated particles

Normally the rate of phagocytosis of complement-IgG-coated particles is at least 30% higher than that of the IgG-coated ones. This ratio for the present controls was 1·57 ± 0·38. The mean corresponding ratios for the psoriasis and PPP groups were 1·29 ± 0·30 and 1·34 ± 0·34 respectively. However, as shown in Fig. 3, the pattern was variable in both groups of patients. Thus in seven of the psoriasis patients the rate of phagocytosis of complement-coated particles was increased > 40%, in eight it was increased by less than 20% and in three it was decreased, compared with the rate for IgG-coated particles. Similarly, among patients with PPP, ten showed an increase of > 40%, seven an increase of less than 20% and one had no increase.

The mean WBC counts were 6·33 ± 1·94 × 10^9/l in the psoriasis group and 6·89 ± 2·30 × 10^9/l in patients with PPP.

Relationship between clinical condition and phagocytic activity

No relation was found in either group between the severity of the disease and the rate of phagocytic uptake of IgG- or complement-IgG-coated particles. The number of vesicles and pustules was thus not correlated with the rate of phagocytosis. There was no difference in phagocytic activity between patients with mild, moderate and severe disease. The only clinical

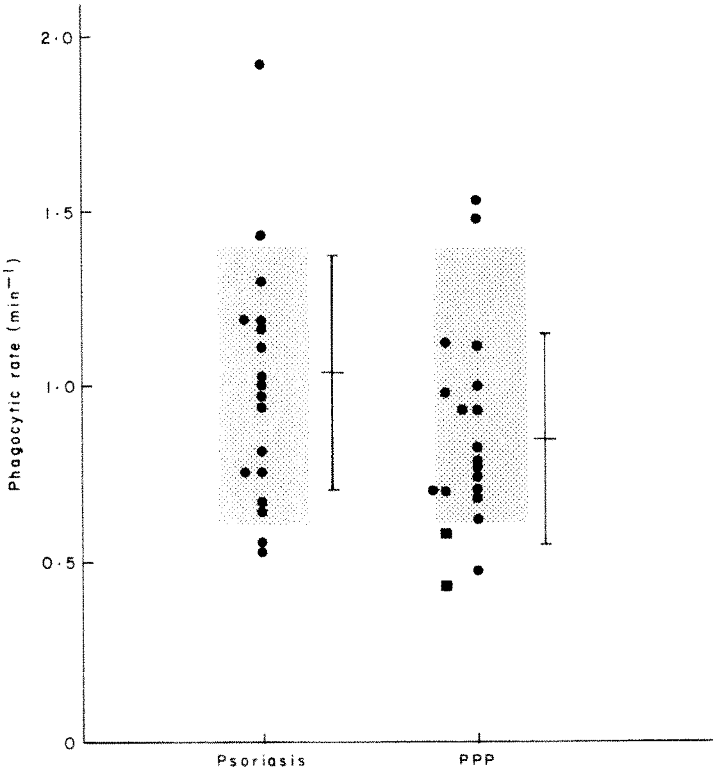

FIGURE 2. The phagocytosis of IgG-C3b-coated particles in buffer medium by PMNs isolated from patients with psoriasis (left) and palmoplantar pustulosis, PPP (right). PPP patients with mild psoriasis ■. The phagocytosis of such particles by normal PMNs is indicated by the shaded areas (95% confidence intervals).

feature possibly related to the phagocytic rate was that the PPP patients with mild psoriasis showed no signs of an increase in this rate. The four patients with arthritis did not differ from the others with regard to the rate of phagocytosis. Nor did the three patients on a low dose (25 mg daily) of etretinate, the three on intermittent PUVA and the one on UV-B differ from the others in their phagocytic rate.

DISCUSSION

In this study the phagocytic performance of granulocytes from patients with psoriasis and PPP was examined by a kinetic method. It allows information on the rate of particle uptake and is based on repeated sampling at short intervals, whereas other models for studies of phagocytes usually determine the uptake at one end-point only.

The phagocytic process is dependent upon several factors within, on and around the granulocyte. A series of experimental data have led to the conclusion that interaction with the Fc-receptor is a prerequisite for efficient uptake of particles by PMNs, whereas opsonization with C3b merely facilitates adhesion of the particle to the cellular membrane, thereby facilitating the subsequent interaction with the Fc-receptor.

The method used in this study has the capacity to discriminate the uptake mediated by the

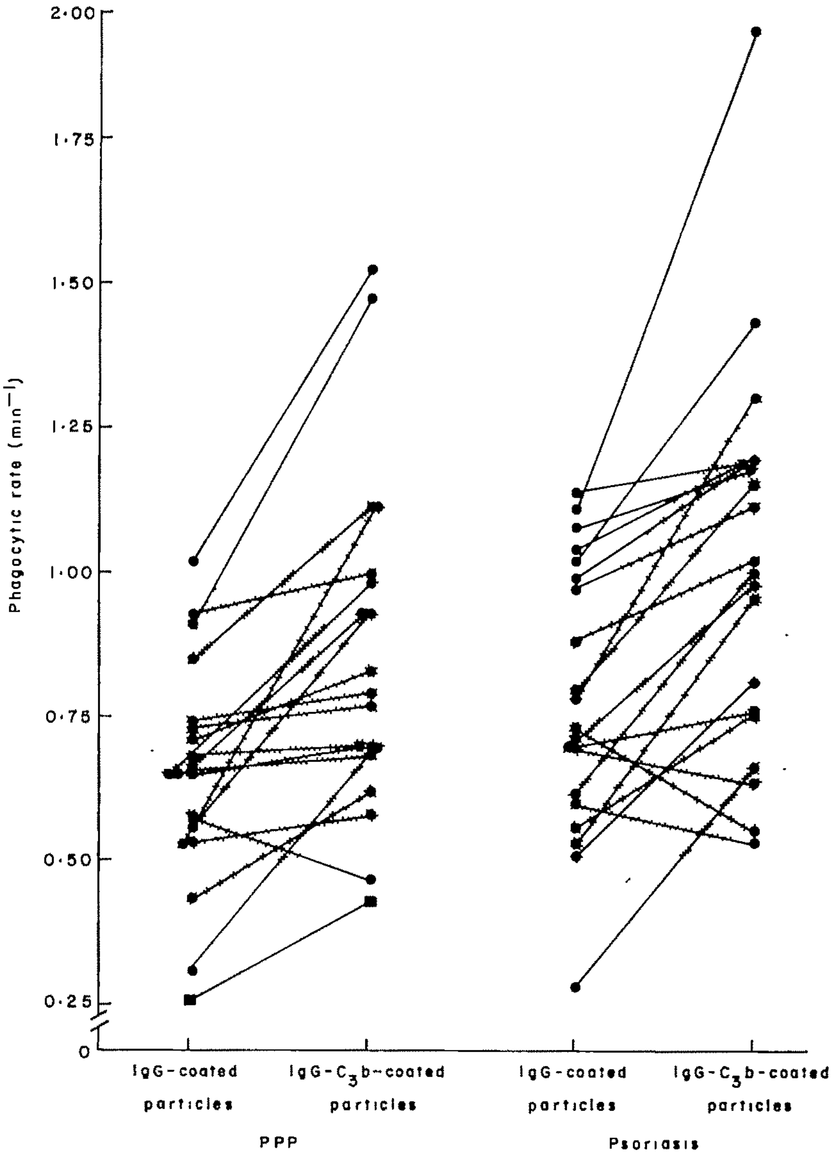

FIGURE 3. The relation between the rates of phagocytosis of IgG-bound and IgG-C3b-coated particles by PMN isolated from patients with PPP and with psoriasis. The shaded area indicates the phagocytosis of such particles by normal PMNs (95% confidence interval).

Fc-receptor (measured with the IgG-coated particles) from that mediated by the C3b receptor (measured with the complement IgG-coated particles).

The Fc-receptor-mediated phagocytosis was enhanced in both groups of patients. This phenomenon has also been observed in some other diseases studied by this method, such as SLE and acute bacterial infections (Hällgren, Håkansson & Venge, 1978), and might be an event secondary to the activity of the underlying disease. Studies of the phagocytic rate with this method in other skin disorders have not yet been done. Several factors with the capacity to enhance the Fc-receptor function of PMNs have been identified. Some of these involve the activities of proteolytic enzymes contained within the primary granules of neutrophils, such as elastase and CCP (chymotrypsin-like cationic protein, cathepsin G). Thus the enhancement may be a result of the release of minute amounts of granule enzymes as a consequence of production of release-promoting activity either locally or within the circulation (Venge *et al.*, 1982). In favour of this hypothesis is the finding of elevated levels of neutrophil granule proteins, e.g. lactoferrin, in the serum of both psoriasis and PPP patients and of a significant relationship between lactoferrin levels and the rate of Fc-receptor-mediated phagocytosis (Lundin *et al.*, unpublished). Another mechanism could be an effect of hyaluronic acid, which has been shown to enhance PMN function both *in vitro* and after administration *in vivo* to humans (Håkansson *et al.*, 1980). Mobilization of hyaluronic acid and other connective tissue constituents from the skin to the blood may be a consequence of the degradation of tissue mediated by inflammatory processes. The dermal inflammation beneath and around the psoriatic lesions is surprisingly intense with perivascular infiltrates of macrophages and lymphocytes (Braun-Falco, 1977) as well as of neutrophils (Chowaniec & Jablonska, 1979) both in the upper reticular and in the papillary layer. If enhancement of the Fc-receptor function is a phenomenon secondary to the disease, a relationship between the severity of the disease and the degree of this enhancement might be expected. We failed to find such a relationship, however, since neither the patients with mild psoriasis nor those with mild PPP differed from the others of their groups with regard to Fc-mediated phagocytic behaviour. This may, of course, have many explanations. One of the most obvious is that both granule enzymes and hyaluronic acid might affect the Fc-receptor function in a dose-dependent fashion, implying a reduction in the enhancement or even an inhibitory effect at higher concentrations, as has been demonstrated *in vitro* (Håkansson *et al.*, 1980). An alternative explanation might be that the enhanced Fc-receptor function is part of an abnormality inherent in psoriasis and PPP and predisposing to the disease.

Normally, as observed in all the controls, further opsonization of IgG particles by means of serum leads to an increased rate of uptake of the particles. C3b has been shown to be the main opsonizer (Schreiber & Fahrney, 1976). However, nearly 50% of the patients in both the psoriasis and the PPP group failed to produce the normal potentiation of the uptake induced by C3b. This may indicate that the C3b receptor function is altered or disturbed. A dysfunction of C3b receptors has also been demonstrated in other inflammatory diseases, such as sarcoidosis (Hällgren *et al.*, 1982). Experimentally, C3b receptor dysfunction can be brought about by treatment of the PMNs with proteolytic enzymes or exposure of the cells to H_2O_2 in combination with myeloperoxidase and a halide such as Cl^-. Both these mechanisms cause the dysfunction *in vitro* at concentrations very likely to occur *in vivo* (Håkansson & Venge, 1982). As discussed above, activation of the PMNs and the triggering of degranulation in both psoriasis and PPP are indicated by elevated serum levels of the granule protein lactoferrin. The seemingly contradictory findings of enhanced Fc-receptor function and a parallel reduction in the C3b-receptor function have their counterparts in experimental data (Håkansson & Venge, 1982).

Granulocytes from psoriasis patients have previously been reported to have subnormal (Bork & Holzmann, 1974; Dubertret, Lebreton & Touraine, 1982), normal (Krueger, Hill & Jederberg, 1978) and increased (Wahba *et al.*, 1978) phagocytic activity. The results of these earlier studies on the phagocytic performance are not directly comparable to those of our study, owing to differences in methods. However, increased monocyte and neutrophil cytotoxicity was recently found in psoriasis patients with more than 25% of the skin surface affected (Herlin, Borregaard & Kragballe, 1982). In that study the antibody-dependent cell-mediated cytotoxicity was assessed with IgG-sensitized human erythrocytes as target cells. It was suggested that the increase in cytotoxicity may have been induced by enhanced Fc-receptor activity, which is compatible with our observations.

Some of our psoriasis patients were under treatment but the phagocytic rates had no tendency to differ in these patients. This does not, of course, exclude a possible influence on the phagocytic rate by active treatment.

There are no comparable studies on granulocyte phagocytosis in PPP. The decreased PMN ingestion of yeast particles reported by Molin & Rajka (1971) might be explained by a C3b dysfunction, as autologous plasma was present in their system. The decreased bactericidal activity of *Staphylococcus aureus* but not of *E. coli* in PPP observed by Mattsby-Baltzar *et al.* (1978) has no obvious link to the results of our study. In both the above two studies PPP patients with signs of psoriasis had been excluded. The number of patients with PPP combined with minimal psoriasis in our study was small, but the difference with regard to the phagocytic behaviour between this group and both the pure PPP group and the psoriasis patients should be further investigated.

In conclusion, this study has demonstrated that in psoriasis both the Fc- and the C3b-dependent phagocytosis differ from the normal granulocytic performance. The increase in Fc-receptor activity is paralleled by a disturbance of C3b function in many of the patients, which might explain the contradictory results in this field. Furthermore, the PPP patients seem to have the same granulocytic behaviour as those with psoriasis, favouring the idea of a possible relationship between these two disorders.

ACKNOWLEDGMENTS

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Detection of interferon in the sera of patients with psoriasis, and its enhancement by PUVA treatment

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SUMMARY

Interferon-like activity could be detected in the sera of nineteen psoriatic patients (interferon titres between 8 and 64 i.u./ml), whereas healthy controls were negative. Following PUVA treatment the interferon levels increased significantly (interferon titres between 32 and 128 i.u./ml). An enhanced interferon production could explain some of the immunological phenomena connected with PUVA treatment.

Interferon (IFN) has been detected in the circulation of patients with auto-immune diseases (Skurkovich & Eremkina, 1975; Hooks *et al.*, 1979; Ohno *et al.*, 1982) and this prompted us to look for IFN activity in the sera of psoriatic patients and to study its response to PUVA therapy. According to Lindner-Frimmel (1974) and Mozes & Vilček (1974), ultraviolet radiation should be capable of potentiating an induced IFN production in human cells.

METHODS

Psoriatic patients

Nineteen psoriatic patients (eleven female and eight male) with an average age of 45 years (range 19–61) were receiving PUVA. The patients had 20% or more body involvement.

PUVA therapy

Treatments were given three times each week for 4 weeks using oral methoxsalen (0·6 mg/kg) and whole body exposure, 2 h later, to UV-A radiation in PUVA unit, as previously described (Meffert, Metz & Sönnichsen, 1978). Every patient received at least six PUVA treatments over a

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period of 2 weeks (3000 J/m^2 to 8000 J/m^2) and twelve treatments over a period of 4 weeks (3000 J/m^2 to 21,000 J/m^2), respectively.

IFN assay

Blood samples were collected by venepuncture before and 2 and 4 weeks after the beginning of PUVA treatment. Sera were stored at −70°C until use. The IFN assay was performed in microtitre plates (Nunc, Aarhus, Denmark) using human embryonic diploid fibroblast cells and vesicular stomatitis virus (strain Indiana, 10^3 plaque-forming units per well) as the challenge virus. Every sample was titrated in duplicate. Results were recorded as 50% reduction doses of the viral cytopathic effect. A laboratory standard calibrated against a National Institute of Health α-IFN standard (G-023-901-527) having 1000 international units (i.u.) per ml was used in each assay.

RESULTS

The sera of all nineteen psoriatic patients which were tested for IFN activity were positive, the titres ranging from 8 to 64 international units per ml as indicated in Fig. 1. These nineteen patients were subjected to routine PUVA therapy and blood samples were taken 2 weeks after the beginning of treatment. As shown, the IFN levels increased following PUVA treatment (32 to 128 i.u./ml).

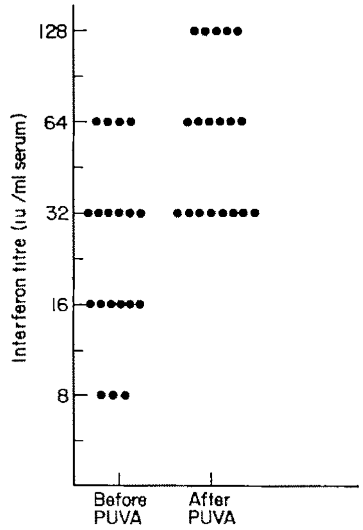

FIGURE 1. Serum interferon level in nineteen patients with psoriasis before and after at least six PUVA treatments. Chi-square test: $P < 0.01$.

In six of these patients the serum IFN level was estimated again after a total of 4 weeks' treatment (twelve sessions). The results are shown in Table 1. In contrast, IFN was undetectable in serum samples from ten healthy controls.

TABLE 1. Serum interferon levels in patients with psoriasis following PUVA treatment. IFN titres (i.u. per ml) before and after six and twelve treatments

	Number of PUVA treatments		
Patient	None	6	12
1	1:16	1:64	1:64
2	1:8	1:64	1:128
3	1:16	1:32	1:64
4	1:32	1:32	1:32
5	1:32	1:128	1:128
6	1:64	1:128	1:128

DISCUSSION

Recent reports confirm the hypothesis that under normal conditions certain cells may have the inherent ability to produce IFN at very low levels (Stewart, 1981), and this might be necessary for normal cellular proliferation. In conditions connected with abnormal cellular proliferation, as in psoriasis, this basal IFN synthesis might be triggered to measurable levels by as yet unrecognized inducer(s), such as endogenous mitogen(s) involved in the mechanism of pathogenesis. In general, no IFN is detectable in the sera of healthy controls (Emödi *et al.*, 1975). Thus the detection of IFN in the sera of psoriasis patients might be associated with defective cellular regulation mechanisms.

On the other hand PUVA treatment elevates the level of serum IFN in psoriatic patients. The potentiating ability of UV radiation with regard to IFN synthesis (Lindner-Frimmel, 1974; Mozes & Vilček, 1974) may result from its action on the cellular membrane rather than its DNA-damaging and other effects, since both the IFN induction and release processes are known to be membrane-dependent.

An enhanced IFN production effected by UV radiation might help to explain several apparently unrelated immunological phenomena connected with PUVA treatment such as prolongation of the survival of full thickness skin grafts in rabbits (Morison *et al.*, 1981), a decrease in the proportion of T cells (E rosetting cells) while the proportion of null cells is increased (Ortonne *et al.*, 1977; Greaves, Price & Vella-Briffa, 1980) and inhibition of cell proliferation in mixed lymphocyte cultures (Lindahl-Kiessling & Safwenberg, 1971; Kraemer *et al.*, 1981) or after mitogenic stimulation (Scherer, Kern & Braun-Falco, 1977; Kraemer *et al.*, 1979).

If these findings are indeed mediated by IFNs, the beneficial effects of PUVA treatment in mycosis fungoides, psoriasis and some other skin disorders might point to a common factor in the pathogenesis of these different conditions. Moreover, it may open the field for applied interferon research, including the clinical application of suitable IFN inducers in certain skin diseases.

ADDENDUM

Since this paper was submitted, interferon has been demonstrated in suction blister fluid from patients with psoriasis (Bjerke *et al.*, 1983).

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Anatomical mapping of epidermal Langerhans cell densities in adults

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SUMMARY

The densities of T6 antigen-bearing Langerhans cells in 112 biopsies of human skin from sixteen surgical out-patients and four cadavers were determined for eight anatomical regions. The regional mean densities (± s.e.m.) of epidermal Langerhans cells per mm^2 were: head and neck, 489 ± 27; chest, 466 ± 22; back, 466 ± 11; upper extremities, 458 ± 25; lower extremities, 431 ± 30; buttocks, 411 ± 11; genitalia, 298 ± 45; soles, 58 ± 12. No statistically significant differences were found between any of these Langerhans cell densities except for that of the soles which was lower than those of all other regions ($P < 0.002$). No significant differences were detected between the mean densities of patients and cadavers, Caucasians and Hispanics or males and females.

Although numerous studies of Langerhans cells have been performed little data is available regarding their quantitative distribution. In their study of guinea-pig epidermis, Wolff & Winkelmann (1967) observed no variation in Langerhans cell density in different anatomical regions (ears, lower back and abdomen) or in different strains, but recent studies have revealed regional differences in epidermal Langerhans cell densities of various rodent species (Bergstresser, Fletcher & Streilein, 1980).

Since Langerhans cells are important for the induction of delayed contact hypersensitivity, we have now studied the regional densities of human epidermal Langerhans cells to establish a baseline for other studies of the effects of diseases or physical and pharmacological agents on these cells.

METHODS

One hundred and twelve specimens of non-lesional skin were obtained from sixteen surgical out-patients and four cadavers. Preliminary time-course experiments revealed no significant

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difference between epidermal Langerhans cell densities of freshly excised newborn human foreskin and foreskin specimens stored at 4°C up to 24 hours. Therefore, specimens obtained within 24 hours post mortem from cadavers refrigerated almost immediately after death were included in this study.

All biopsies were 4 mm in diameter and, in the case of surgical out-patients, were excised following subcutaneous injection of 1% lignocaine with 1:1000 epinephrine.

Subjects

Cadavers. Eighteen to twenty-three selected sites were biopsied from each cadaver. We were unable to obtain specimens from the head, neck and hands because of legal restrictions.

Case 1. A 77-year-old Hispanic man with a history of diabetes mellitus developed an acute myocardial infarction with subsequent sudden death (Fig. 1A).

Case 2. A 59-year-old white woman with a history of atherosclerotic heart disease and congestive heart failure developed a lateral myocardial infarction with subsequent cardiogenic shock and death (Fig. 1B).

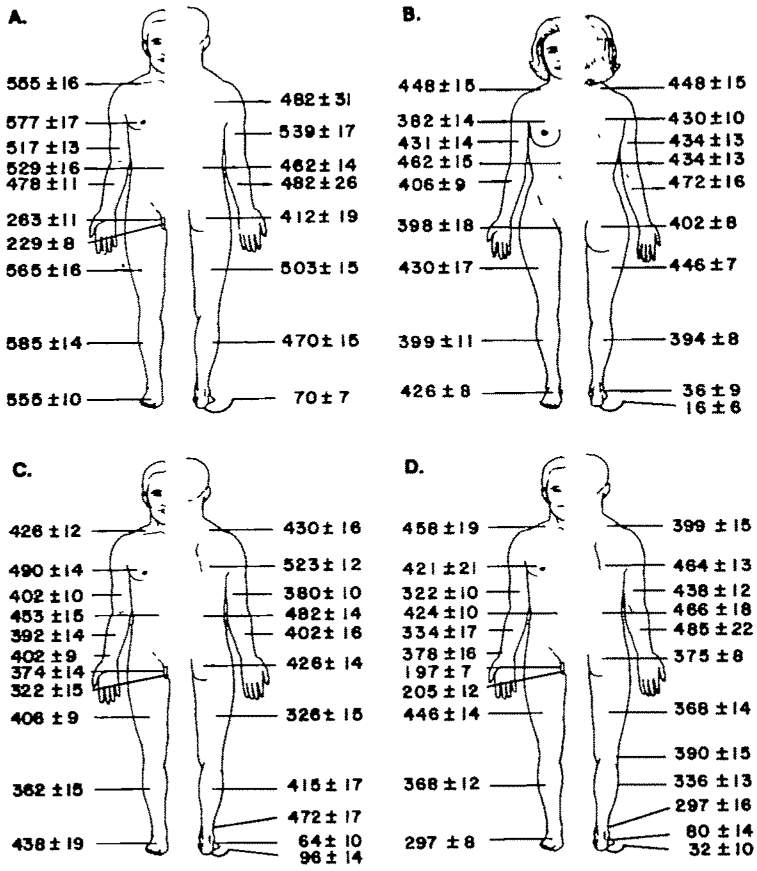

FIGURE 1. Epidermal Langerhans cell densities (mean ± s.e.m./mm^2) in biopsy specimens of selected anatomical sites of cadavers. A. Case 1, B. Case 2, C. Case 3 and D. Case 4.

Case 3. An 86-year-old white man with a history of chronic obstructive pulmonary disease expired following acute respiratory failure (Fig. 1C).

Case 4. A 32-year-old white man with a history of intravenous drug abuse and alcoholism succumbed to progressive hepatic failure (Fig. 1D).

Surgical out-patients. Eight Hispanic and eight Caucasian surgical out-patients, 26 to 61 years of age, with no history of significant medical illnesses and receiving no medications (Fig. 2) were included in this study. Ten patients were female and six were male.

Antibody

Fluorescein isothiocyanate-conjugated mouse monoclonal anti-human T6 antigen IgG1 (Ortho Pharmaceutical Corp., Raritan, N.J.) was used throughout. This has been shown to react with 70% of human thymocytes and with epidermal Langerhans cells (Fithian *et al*., 1981; Chu *et al*., 1982) but not with peripheral mononuclear cells (Kung *et al*., 1979). A 1:25 dilution of the antibody in sterile minimum essential medium with Earle's salts and 5% bovine serum albumin was employed, detecting the same number of epidermal Langerhans cells as undiluted antibody.

Preparation of epidermal sheets

Fat-trimmed tissue specimens were immersed in 1 N sodium bromide for 30 min at 37°C. The epidermis was subsequently teased off the dermis with fine forceps and washed in phosphate-buffered saline prior to immunofluorescent staining.

Immunofluorescent detection of epidermal Langerhans cells

Epidermal sheets were incubated in fluoresceinated mouse monoclonal anti-human T6 antigen IgG1 for 1 h at 37°C in a humidified incubator and subsequently washed five times with cold phosphate-buffered saline. The specimens were mounted in glycerol gelatin between glass

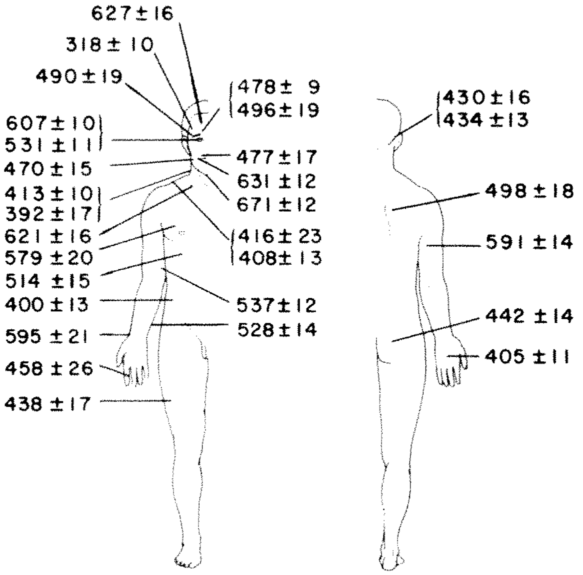

FIGURE 2. Epidermal Langerhans cell densities (mean ± s.e.m./mm²) in biopsy specimens of selected anatomical sites of sixteen surgical out-patients (composite).

coverslips and fluorescing dendritic cells were enumerated under epifluorescent microscopy. Between ten and twenty randomly chosen, non-overlapping fields (0·0248 mm^2 usually containing approximately 12 T6+ cells) were examined, and the mean number of Langerhans cells ± s.e.m. per mm^2 calculated.

In preliminary experimentation to determine if incubation with 1 N sodium bromide affects subsequent detection of T6 antigen-bearing cells, a biopsy of breast skin was bisected and the 4-mm halves incubated at 37°C for 30 min in either phosphate-buffered saline or sodium bromide and stained for T6+ cells as described above. The mean number ± s.d. of fluorescing T6+ cells in twenty 400 × fields of 6-micron thick cryostat sections of the saline and sodium bromide treated halves were not significantly different, being 7·65 ± 1·18 and 7·90 ± 1·16, respectively.

Statistical analyses. Student's t test comparisons were performed and only P values less than 0·002 were considered significant due to multiple inter-regional comparisons.

RESULTS

The mean number of epidermal Langerhans cells (± s.e.m.) per mm^2 in specimens from different sites is shown (Figs 1 and 2). Since the greatest number of cadaver biopsy specimens were from the trunk (back and chest), this mean density in this area was compared with that from the corresponding area of live patients.

No significant differences were found in mean truncal Langerhans cell densities between Hispanic patients (502 ± 42) and the Hispanic cadaver (521 ± 48), nor between Caucasian patients (446 ± 34) and Caucasian cadavers (447 ± 5).

The specimens obtained from cadavers and live patients were assigned to one of eight anatomical regions, and the mean densities of epidermal Langerhans cells of each region calculated (Table 1). Epidermis from the soles contained significantly fewer Langerhans cells (58 ± 12, $P < 0·002$) than any other region. Although the genitalia contained fewer Langerhans cells (298 ± 45) than the head and neck, chest, upper extremities, lower extremities and buttocks, the differences were not statistically significant.

The mean number of Langerhans cells ± s.e.m. per mm^2 was 507 ± 34 within the epidermis from truncal regions of Hispanic individuals and 446 ± 15 within the corresponding region of Caucasian subjects. These densities are not statistically different. In addition, race- and sex-matched cadavers of 32 and 86 years of age, respectively, did not significantly differ in their mean truncal (439 ± 28; 455 ± 48, respectively) or in their mean total body (322 ± 50; 366 ± 47) Langerhans cell densities. Males and females did not differ significantly in their mean truncal Langerhans cell densities.

DISCUSSION

Variations in regional densities of rodent epidermal Langerhans cells as detected by ATPase activity have been reported by Bergstresser *et al.* (1980). They found significantly lower densities within the hamster cheek pouch when compared with specimens from the ear, back, foot pad and buccal mucosa. The density of epidermal Langerhans cells of the cornea of the hamster (MHA and CB inbred strains), mouse (C57 BL/6J, A/Jax and BALB/c nu/nu) and guinea-pig (albino and red outbred strains) and of the tail of the mouse were also found to be less than within the other aforementioned anatomical sites. This and other investigations suggest

TABLE 1. Epidermal Langerhans cell densities in eight anatomical regions

Anatomical region[a]	Number of subjects	Number of biopsies	Mean number of Langerhans cells[b] ± s.e.m./mm²
Head and neck	13	15	489 ± 27
Chest	9	18	466 ± 22
Back	5	12	466 ± 11
Upper extremities	7	24	458 ± 25
Lower extremities[c]	5	5	431 ± 30
Buttocks	5	5	411 ± 11
Genitalia[d]	4	7	298 ± 45
Soles	4	7	58 ± 12

[a] Four-mm diameter skin biopsies were obtained from sixteen surgical out-patients and four cadavers.

[b] T6 antigen-bearing Langerhans cells were counted under epifluorescent microscopy and an average Langerhans cell density for each region for each subject was used to calculate the mean density for each region.

[c] Excluding the soles.

[d] Shaft of penis, scrotum and labia majora.

that a local paucity of Langerhans cells reduces or modifies the processing and presentation of antigens coming in contact with these sites (Bergstresser *et al.*, 1980; Toews, Bergstresser & Streilein, 1980; Streilein, Toews & Bergstresser, 1980). This contention is supported by the hyporesponsiveness of transplanted tail grafts of C57 BL/6 mice sensitized with dinitrofluorobenzene, suggesting that Langerhans cell density determines the induction of contact hypersensitivity (Toews *et al.*, 1980; Streilein *et al.*, 1980). Increased numbers of Langerhans cells have been reported in lichen planus, a disease in which enhanced cell-mediated hypersensitivity has been implicated (Bhan *et al.*, 1981; Ragaz & Ackerman, 1981). Conversely, we have recently reported lower Langerhans cell densities in epidermis of anergic patients with sarcoidosis than in control epidermis (Fox *et al.*, 1983).

Friedmann (1981) stained suction blister tops from the anterior forearms of six volunteers and reported the mean (± s.e.m.) density of Langerhans cells to be 730 (60)/mm². This value is greater than that calculated for the upper extremity, 458 (25), in this study in which T6 antigenicity was the Langerhans cell marker. Unlike T6 antigens which, within epidermis, are specific for both Langerhans and indeterminate cells, ATPase activity is present to some degree in all epidermal cells, and might stain 'passenger leukocytes' as well as Langerhans cells. We examined the twenty-four upper extremity biopsies at higher magnification than that used by Friedmann, in order to count only Langerhans cells and to exclude their dendritic processes. The lower specificity, greater subjectivity, limited number of specimens tested, and the possible inclusion of dendrites in Friedmann's study may have resulted in an overestimation of Langerhans cells. On the other hand, the possibility exists that although immunofluorescent detection of T6 antigenicity is specific for epidermal Langerhans cells, it may not detect all such cells.

Our data reveal significantly lower densities within the epidermis of the soles than in any other region tested. These lower densities were not due to an inability to detect Langerhans cells through thick stratum corneum, since the numbers of these cells were also markedly diminished in cross-sectional specimens (unpublished data). It is clinically recognized that allergic contact dermatitis of the foot usually involves the dorsum more commonly than the sole. Although this finding has generally been attributed to the possibility of reduced antigen penetration due to a thickened stratum corneum, our findings may suggest reduced ability to present antigen by Langerhans cells.

Epidermal Langerhans cell densities calculated for corresponding regions from Caucasian and Hispanic individuals did not differ significantly. Further investigations employing greater numbers of subjects are necessary to substantiate these findings and to determine any racial differences.

ACKNOWLEDGMENTS

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Impaired lymphocyte cyclic adenosine monophosphate responses in atopic eczema

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SUMMARY

Peripheral blood lymphocyte cyclic AMP responses to isoprenaline, prostaglandin E2 and histamine were examined in patients with atopic eczema, in the presence and absence of a potent phosphodiesterase inhibitor (PDEI). Basal cyclic AMP levels were not significantly different in atopic and control groups. In the presence of the PDEI, there were impaired cyclic AMP responses to both isoprenaline and prostaglandin E2 in the atopic group. Differences between atopic and control cyclic AMP responses were exaggerated by the omission of the PDEI, when impaired responses to all agonists were observed in lymphocytes from atopic subjects. These results imply that, in atopic eczema, lymphocytes exhibit impaired responses not only at the beta-adrenoceptor site but at unrelated sites of adenylate cyclase activation, and these observations are consistent with increased leukocyte phosphodiesterase activity.

Following the proposal by Szentivanyi (1968) that impaired beta-adrenergic reactivity is a primary determinant of atopy, numerous efforts have been made to substantiate this hypothesis. Studies of peripheral blood leukocytes in atopic eczema have frequently demonstrated impaired adrenergic reactivity as revealed by (i) a loss of regulatory effects on lysosomal enzyme secretion and reduced formation of cyclic adenosine monophosphate (cyclic AMP) (Reed, Busse & Lee, 1976), (ii) decreased affinity of binding sites for radiolabelled beta-adrenoceptor agonists (Pochet, Delespesse & Demaugeuge, 1980) and (iii) an increased ratio of alpha-adrenoceptor to beta-adrenoceptor binding sites (Szentivanyi *et al.*, 1980). In some studies, normal reactivity to prostaglandin E1 has accompanied reduced reactivity to beta-adrenoceptor stimulation (Reed *et al.*, 1976). However, the selectivity of this impaired reactivity can be disputed, since it has been reported that cyclic AMP responses of mononuclear leukocytes are reduced in atopic eczema, not only to beta-adrenoceptor stimulants, but also to histamine (Busse & Lantis, 1979) and to prostaglandin E1 (Parker, Kennedy & Eisen, 1977). Such non-selective impairment of

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responses may be secondary to increased cyclic AMP–phosphodiesterase activity observed in mononuclear leukocytes in atopic eczema (Chan *et al.*, 1981).

We have compared the effects of isoprenaline, histamine and prostaglandin E2 on cyclic AMP production in peripheral blood lymphocytes both in the presence and absence of a potent phosphodiesterase inhibitor (PDEI) to establish whether there is selective impairment of the response to beta-adrenoceptor agonists.

METHODS

Fourteen patients with active atopic eczema (seven males and seven females, mean age 20 years) and fourteen normal volunteers (seven males and seven females, mean age 27 years) were studied. The diagnosis of atopic eczema was established by standard clinical criteria including physical examination, a positive personal history and/or family history of atopy, elevated serum IgE concentration and histopathological features. The extent of skin involvement was defined by use of a '20 area' severity assessment chart, details of which have been reported previously (Zachary & MacDonald, 1983). By this assessment there were five patients with mild, five with moderate and four with severe atopic eczema. Prior to investigation, none had received systemic therapy (e.g. antihistamines) for a period of at least 4 days, although some had received limited application of topical corticosteroids. It was considered to be unethical to withold treatment completely in the more severely affected patients. None had received adrenergic drugs. Normal volunteers were not atopic and were taking no concurrent medications.

Venous blood samples were collected between 9 and 10 a.m., heparinized, diluted with buffer and layered on to Ficoll-Paque. After centrifugation, the mononuclear cell layer was removed, suspended in Hank's buffer and the cells counted. At least 90% of the cell population were lymphocytes, with the remainder being mainly monocytes.

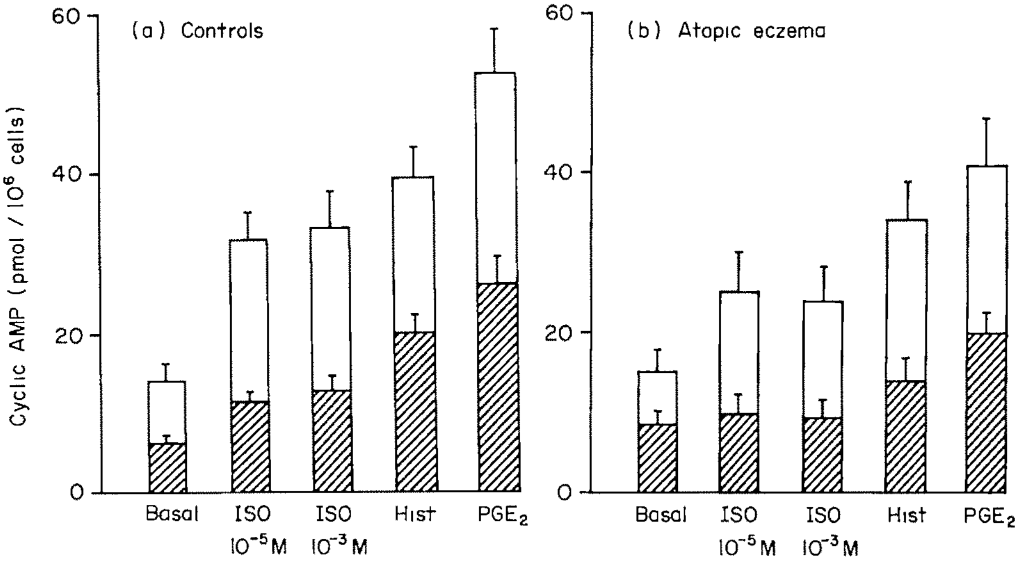

FIGURE 1. Mean cyclic AMP levels (pmol/10^6 cells ± s.e.m.) for unstimulated (basal) samples, and after stimulation with isoprenaline (10^{-5} M and 10^{-3} M), histamine (10^{-4} M) and prostaglandin E2 ($1{\cdot}1 \times 10^{-4}$ M), in the presence □ and absence ▨ of the phosphodiesterase inhibitor (ICI 63197) in (a) control subjects and (b) patients with atopic eczema.

Duplicate aliquots (10^6 cells/ml) were stimulated, in the presence and absence of a potent phosphodiesterase inhibitor (ICI 63197) at 37°C for 10 min. Freshly prepared agonists were added to incubation tubes to the following final concentrations: isoprenaline (10^{-5} M and 10^{-3} M), histamine (10^{-4} M), and prostaglandin E2 ($1{\cdot}1 \times 10^{-4}$ M). Samples without added agonist were included to provide basal cyclic AMP levels. Tubes were immersed in boiling water for 10 min to terminate the response and stored at −20°C. Total cyclic AMP levels were determined, in duplicate, by radioimmunoassay.

Student's *t*-test was employed for statistical comparison, unpaired observations being used for comparison between groups and paired observations for within-subject analysis.

RESULTS

There were no significant differences between basal cyclic AMP levels in cells from atopic and control groups and, as anticipated, higher cyclic AMP levels were recorded in both groups in the presence of the phosphodiesterase inhibitor (Fig. 1). In normal subjects, each agonist produced a significant elevation of cyclic AMP ($P < 0{\cdot}0005$), and responses were more pronounced in the presence of the phosphodiesterase inhibitor (Fig. 1a). Corresponding data for patients with atopic eczema showed an essentially similar pattern, although the magnitude of responses to stimulation was generally less than in the control group (Fig. 1b).

The differences between the atopic and control groups can more readily be appreciated, when responses are expressed as net cyclic AMP response to stimulation (that is, the mean of stimulated less basal cyclic AMP levels). In the presence of the phosphodiesterase inhibitor, responses in cells from atopic patients were significantly less than in cells from normal subjects for both concentrations of isoprenaline and for prostaglandin E2 (Fig. 2). In the absence of the phosphodiesterase inhibitor, the impairment of cyclic AMP responses to isoprenaline and prostaglandin E2 in cells from atopic patients was more pronounced, responses to all three agonists, including histamine, being significantly less than in cells from normal subjects (Fig. 3).

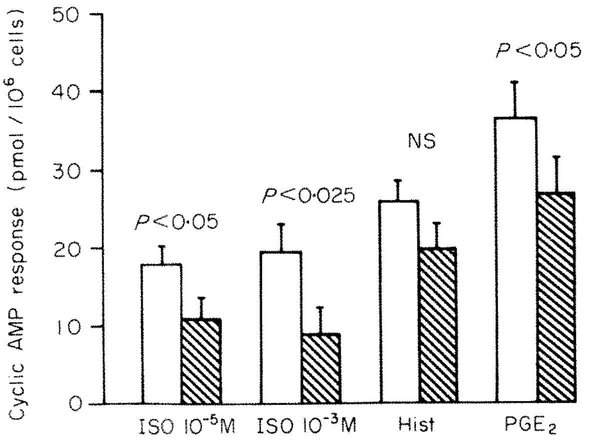

FIGURE 2. Cyclic AMP responses: mean of stimulated less basal levels (pmol/10^6 cells ± s.e.m.) in atopic ▨ and control ☐ subjects in the *presence* of the phosphodiesterase inhibitor.

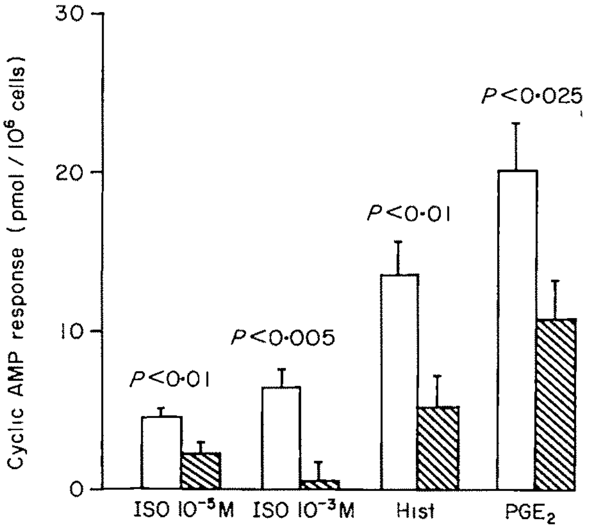

FIGURE 3. Cyclic AMP responses: mean of stimulated less basal levels (pmol/10^6 cells ± s.e.m.) in atopic ▨ and control ☐ subjects in the *absence* of the phosphodiesterase inhibitor.

DISCUSSION

The observation that patients with atopic eczema exhibit impaired cyclic AMP responses of peripheral blood lymphocytes to prostaglandin E2 and histamine, as well as to isoprenaline, implies that impaired reactivity is not confined to the beta-adrenoceptor, but lies at a site common to all three agonists. The present study does not exclude the possibility that there is an impairment of adenylate cyclase activation. However, differences between cells from atopic patients and normal subjects were exaggerated by the omission of a phosphodiesterase inhibitor, and therefore could be a consequence of increased leukocyte phosphodiesterase activity which has been reported in atopic eczema (Chan *et al.*, 1981). It is reasonable to attribute these abnormalities to atopic eczema and not to cutaneous inflammation *per se*, for in a parallel study of twelve patients with psoriasis, lymphocyte cyclic AMP responses to all stimulants were normal (Hanson *et al.*, 1983).

In atopic asthma, impaired leukocyte cyclic AMP responses to isoprenaline have been frequently observed but are often attributed to prolonged beta-adrenoceptor agonist therapy (Greenacre, Schofield & Connolly, 1978; Galant *et al.*, 1978). In the present investigation, particular care was taken to ensure that patients had no previous history of adrenergic medication, so that drug-induced tachyphylaxis can be discounted. It is unlikely that the application of mild topical corticosteroids in the four patients with severe atopic eczema produced systemic effects. On the basis of *in vitro* studies (Davis & Lefkowitz, 1980), steroid therapy would be expected to increase beta-adrenoceptor density and therefore could not contribute to the differences observed between the two groups.

Pre-incubation of mononuclear leukocytes from normal subjects with histamine, prostaglandin E1 or isoprenaline has been reported to cause impaired cyclic AMP responses when the cells are subsequently re-stimulated with these agonists (Safko *et al.*, 1981) and it has been suggested that the impairment of leukocyte cyclic AMP responses in atopic eczema is due to chronic exposure to histamine and to other mediators. This is an attractive hypothesis, although elevation of plasma histamine levels has been observed only in severely affected patients as an inconstant and transient finding (Hanifin, 1977; Ring *et al.*, 1979). In this study, there was no correlation between clinical grading of atopic eczema and impairment of lymphocyte cyclic AMP responses.

Impaired beta-adrenergic reactivity in atopy may reflect the presence of a circulating factor capable of blocking beta-adrenoceptor binding. There have been two independent reports that in the serum of patients with atopic asthma there exists a factor, or factors, which inhibit the specific binding of a radioligand (radiolabelled beta-adrenoceptor antagonist) to the beta-adrenoceptors of lung membrane preparations (Venter, Fraser & Harrison, 1980; Basran *et al.*, 1981). The conclusion that this factor is an antibody (Fraser, Venter & Kaliner, 1981) awaits confirmation. In atopic eczema, the occurrence of a beta-adrenoceptor auto-antibody would not explain the non-selective impairment of lymphocyte cyclic AMP responses demonstrated in the present study.

Impaired beta-adrenergic reactivity in peripheral blood lymphocytes from patients with atopic eczema has been confirmed but this is not selective, since impaired cyclic AMP responses to both histamine and to prostaglandin E2 have also been established.

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The stimulation of hair growth in the flank organs of female hamsters by subcutaneous testosterone propionate and its inhibition by topical cyproterone acetate: dose–response studies

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SUMMARY

A dose-dependent increase in the mass of flank organ hair was produced in 11-week-old female hamsters by subcutaneously injected testosterone propionate. The mass of androgen-stimulated flank organ hair was decreased bilaterally in a dose-dependent manner by cyproterone acetate applied topically to one flank organ of each hamster.

The flank organs of the Syrian Golden Hamster (*Mesocricetus auratus*) have been used extensively for the study of the stimulation and inhibition of sebaceous secretion by androgens and antiandrogens (Burdick & Hill, 1970; Frost & Gomez, 1972). These organs are also the site of other targets of androgen action, namely, the hair follicles and melanocytes (Hamilton & Montagna, 1950). The aggregation within a single organ of three androgen-sensitive cell types, each responding to stimulation by the synthesis of a different measurable product of terminal differentiation, offers an opportunity for investigators to study the factors which influence the specificity of the response and the differential sensitivity of target tissues to hormonal stimuli. The differential sensitivity of flank organ tissues has been demonstrated recently by Vermorken, Goos & Wirtz (1982) who have presented evidence that the pigment and the sebaceous tissue of the flank organ respond independently to hormonal stimuli. Studies of this kind may lead to the development of androgens and antiandrogens which possess a high degree of specificity for a single target cell type and a minimal effect on other targets.

The study reported here demonstrates a procedure by which one of the responses to androgen stimulation, the growth rate of flank organ hair, can be quantified gravimetrically. In addition,

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dose–response data for the stimulation of hair growth by subcutaneously administered androgen and for its inhibition by antiandrogen are presented for the first time.

METHODS

Female Syrian Golden Hamsters [Lak: LVG (SYR) (Outbred)], all having the same birth date, were purchased from Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts, U.S.A. They were fed *ad libitum* with Charles River Rat, Mouse and Hamster Diet RMH-3000, watered from an automatic watering system, and housed in a room with a 12-hour light cycle and a constant temperature of 22°C ± 1. At the start of the experiment, the hamsters were 11 weeks old and weighed 93 ± 19 g (mean ± s.d.). On each of 5 successive days, the dorsal hair on and around the flank organs of ten animals was clipped with electric clippers, and the stubble was removed with Surgex Hair Remover Cream (Cooper Laboratories, Wayne, N.J., U.S.A.). The hamsters were under ether anaesthesia during this procedure, as well as during subsequent treatments. Each group of ten hamsters received one of the following doses of testosterone propionate (TP) (Research Plus Laboratories, Denville, N.J., U.S.A.) per animal in 0·05 ml of peanut oil (Planters, Standard Brands, Inc., New York, N.Y., U.S.A.) injected subcutaneously in the dorsal neck or scapular region: 400 μg, 135 μg, 45 μg, 15 μg, 0 μg. Injections were given daily, except weekends, for 3 weeks. The hamsters were killed on the 22nd day after the initial treatment day by carbon dioxide inhalation.

All of the pelage hair within 1 cm of the regrown flank organ hair was cut as close to the skin as possible with fine scissors. The TP-stimulated flank organ hair, distinguished by its coarseness and pigmentation from the pelage hair, was then plucked with wax (Zip Wax, Colorado Chemical Company, Roberts Proprietaries, Inc., New York, N.Y., U.S.A.). The hair was recovered by dissolving the wax in xylene and trapping the hair on humidity-equilibrated, tared, glass fibre filters. After the hairs had been washed free of wax and the residual xylene had been evaporated, the hairs and filters were again humidity-equilibrated (relative humidity 61% ± 1) and weighed.

Hamsters used to determine the effect of topical cyproterone acetate (CA) (recrystallized from Androcur tablets, Schering A.G. Berlin/Bergkamen) on flank organ hair growth in TP-stimulated animals were 12 weeks old at the start of the experiment and weighed 115 ± 7 g (mean ± s.d.). These hamsters were stimulated with a subcutaneous injection of 200 μg of TP

TABLE 1. Hair growth stimulation on the flank organs of female hamsters following subcutaneous injection of graded doses of testosterone propionate (TP) in peanut oil 5 days/week for 3 weeks

	Flank organ hair mass (mg) (mean ± s.e.)		
TP dose (μg)	Left	Right	n
0	1·38 ± 0·19	1·91 ± 0·39	10
15	1·31 ± 0·09	1·61 ± 0·15	10
45	2·89 ± 0·23	3·01 ± 0·26	10
135	4·76 ± 0·47	5·29 ± 0·47	10
400	9·27 ± 1·05	8·89 ± 0·97	10

daily, except weekends, for 3 weeks, a dose estimated by probit analysis to produce at least a doubling of the flank organ hair mass in 97% of the test animals. Immediately following injection, each hamster received a topical treatment of CA in 50 μl of acetone on one flank organ and 50 μl of acetone only on the contralateral flank organ. Each experimental group of ten hamsters received one of the following doses of CA: 900 μg, 300 μg, 100 μg, 0 μg. The control group received no TP and the acetone vehicle only.

RESULTS

Effect of subcutaneous testosterone propionate

Subcutaneous TP in the dose range from 0 to 400 μg/day, 5 days/week for 3 weeks, increased the mean mass of coarse flank organ hair in a dose-dependent manner from 1 or 2 mg to 9 mg per flank organ in female hamsters (Table 1). A logarithmic-probability analysis (Litchfield & Wilcoxon, 1949) of the data for the percentage of sites which exhibited a doubling of the control hair mass determined that a subcutaneous TP dose of 200 μg/day produced at least a doubling of the hair mass in 97% of treated hamsters (Fig. 1). Accordingly, this dose was selected to stimulate hair growth in the subsequent experiment on the effect of topical CA upon flank organ hair growth.

Effect of topical cyproterone acetate

When female hamsters were stimulated with 200 μg TP/day, topical CA in the dose range from 0 to 900 μg/day, 5 days/week, for 3 weeks reduced the mean hair mass of both the CA-treated and the acetone-treated flank organs from 6 mg to 2·5 mg per flank organ in a dose-dependent manner (Table 2). This observation indicates that the CA was absorbed and produced an equivalent effect on the vehicle-treated flank organ.

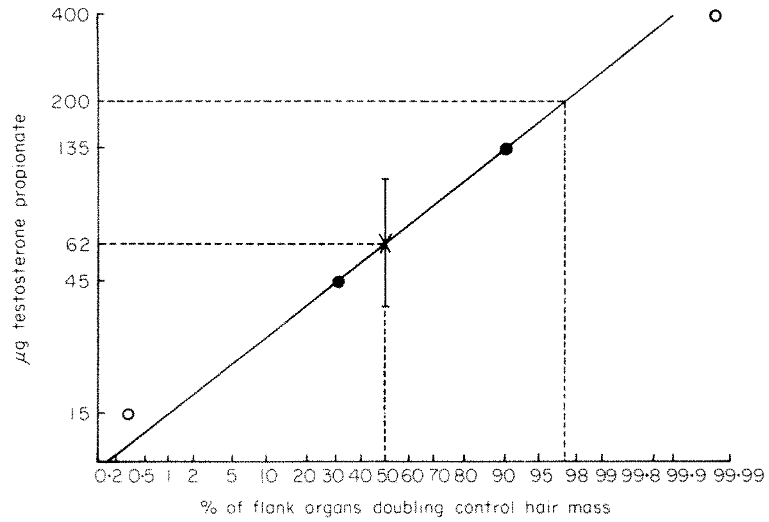

FIGURE 1. Logarithmic-probability plot of subcutaneous testosterone propionate dose vs. percentage of flank organs of female hamsters in each dosage group which at least doubled the mean hair mass of the flank organs of the vehicle-injected control group. Doses injected daily 5 days/week for 3 weeks. Open circles are corrected values for 0% and 100% effects (Litchfield & Wilcoxon, 1949). Median effective concentration was 62 μg. A 200 μg dose was used to stimulate the hamsters in the inhibition study.

TABLE 2. Hair growth inhibition on the flank organs of testosterone propionate (TP)-stimulated female hamsters following unilateral topical application of graded doses of cyproterone acetate (CA) in 50 μl of acetone to flank organs 5 days/week for 3 weeks

		Flank organ hair mass (mg) mean ± s.e.		
CA dose (μg)	TP dose (μg)	Acetone site	CA site	n
0	0	1·15 ± 0·06	1·14 ± 0·11	10
0	200	6·23 ± 0·54	6·09 ± 0·70	10
100	200	3.36 ± 0·08	3·34 ± 0·11	9
300	200	3.16 ± 0·29	3·11 ± 0·42	9
900	200	2·46 ± 0·24	2·55 ± 0·32	10

DISCUSSION

Among the criteria which have been used to evaluate an antiandrogenic effect in the hamster flank organ are:

(1) 'diameter of palpable bulk and pigmentation' (Gomez & Frost, 1975)
(2) a subjective comparison of 'size and pigmentation' (Voigt & Hsia, 1973)
(3) the 'longest diameter of the organs' (Vermorken *et al.*, 1980)
(4) 'flank organ weight' and '^{14}C[acetate]-incorporation into flank organ lipids' (Lutsky *et al.*, 1975)
(5) a development score based on a subjective, integrated estimate of 'degree of pigmentation, coarseness of hair stubble, area and elevation' (Chakrabarty *et al.*, 1980).

As Goos, Wirtz & Vermorken (1982) have pointed out in the discussion of their complex histometric method for evaluating antiandrogens, '. . . visual observation of the pigmented spot may give an incomplete impression of the effectiveness of . . . an antiandrogen,' and '. . . visual observation alone of the hamster flank organ may lead to faulty conclusions about the size and activity of the sebaceous glands'.

Gomez & Moskowitz (1980) have recognized that the flank organs contain three androgen-sensitive tissues, the responses of which can be measured independently. These are the sebaceous gland basal cells, the dermal and follicular melanocytes, and the hair follicle matrix cells. The proximity of three androgen target tissues within the flank organ presents an opportunity to study the factors which mediate the independence and interdependence of their responses to identical stimuli. Such studies may lead to the development of androgens and antiandrogens with a high degree of specifity for a single target tissue and thereby minimize undesirable effects on other potential target tissues. In the study reported here we have concentrated on quantifying the hair growth response to androgenic stimulation and its inhibition by an antiandrogen. A subcutaneous stimulating dose of TP which is large enough to produce a measurable effect on hair growth, but not so large as to mask the antagonistic effect of a weak antiandrogen has been established by probit analysis of dose–response data. CA, which has been shown to inhibit androgen-dependent hair growth in hirsutism by oral administration (Ebling *et al.*, 1977; Peereboom-Wynia & Beek, 1977; Jones *et al.*, 1981), was shown to act similarly on hamster flank organ hair following topical application. Dose–response data for hair growth inhibition by a series of antiandrogens will permit the comparison of the relative efficacy of antiandrogens against a standard androgenic stimulus.

In this study, CA was demonstrated to reduce androgen-dependent hair growth in the hamster flank organ by topical application. The effect is dose dependent and bilateral, although only one flank organ of each animal was treated. This result appears similar to the bilateral effect of the unilateral application of the antiandrogen, flutamide, upon the flank organ weight and [^{14}C]acetate incorporation into lipid in TP-stimulated female hamsters and on flank organ weight in male hamsters (Lutsky *et al.*, 1975).

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Pharmacology and Treatment

Transfusion therapy for erythropoietic protoporphyria

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SUMMARY

Five patients with erythropoietic protoporphyria, in whom beta-carotene treatment had proved unsuccessful, were treated on 5 or 6 occasions with washed, packed red blood cell transfusions. Following the transfusions, the photosensitivity and the protoporphyrin levels in the red blood cells decreased considerably. This treatment may be useful in reducing the high protoporphyrin level in patients with a rapidly deteriorating hepatic function.

Erythropoietic protoporphyria (EPP) is characterized by markedly elevated levels of the haem precursor protoporphyrin (PP) in the erythrocytes, plasma and stools. Clinically, most cases of EPP are manifested by a photosensitive dermatosis. Erythema, oedema and thickening of sun-exposed skin are common, while blistering and scarring are rare. EPP is usually considered a mild disease, but the increased incidences of cholelithiasis and fatal liver disease suggest that, at least in some patients, EPP is not a benign condition (Mathews-Roth, 1980).

In the past, the treatment of EPP consisted of the administration of antihistamines, topical sunscreens, anti-malarials and vitamins. Recently, beta-carotene in high oral doses has been shown to be an effective treatment for EPP (DeLeo *et al.*, 1976; Mathews-Roth, 1977; Mathews-Roth *et al.*, 1977). However, the current methods of treatment are not entirely satisfactory.

Bechtel, Bertolone & Hodge (1981) observed an 8-year-old boy with classic EPP, in whom a notable decrease in photosensitivity symptoms developed after several transfusions with washed packed red blood cells (RBCs) and the reduction in photosensitivity was associated with a decline in free erythrocyte PP levels.

We now describe five patients suffering from EPP who were treated by RBC transfusions. These administrations resulted in a decline in PP levels, associated with a significant suppression of photosensitivity symptoms.

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TABLE 1. Demographic data and clinical features of 5 patients with erythropoietic protoporphyria

Case No.	Age (yr) and sex	Age at onset (yr)	Burning	Pruritus	Pain	Oedema	Erythema	Scarring
1.	21, M	1	+	+	−	+	+	+
2.	24, F	1	+	+	−	+	+	+
3.	9, M	5	+	+	−	+	+	−
4.	14, M	2	+	+	−	−	+	−
5.	22, M	4	+	+	+	+	+	−

METHODS

The study was performed in five EPP patients in whom beta-carotene therapy had proved to be of little or no benefit. The most important clinical features are listed in Table 1, and Table 2 gives the main laboratory data and the tolerance to sunlight. The porphyrins were extracted from erythrocytes by the method of Heller, Labbe & Nutter (1971), and were chromatographed as described by Doss (1972). Gallstones were not found in any of the patients, and the liver function tests, including GOT, GPT, gamma-GT and serum bilirubin levels, were found to be in the normal ranges. The alkaline phosphatase activity was an exception: in patients 3 (50 U/l) and 4 (88 U/l) this somewhat exceeded the upper limit of the normal range (48 U/l).

Patients 1 and 2 were siblings, and patients 3 and 4 were cousins on the paternal side.

Prior to the treatment, the patients and also the mothers of patients 3 and 4 were informed of the risks, which included transfusion reactions, hepatitis and hypersideraemia.

The transfusion therapy was performed with washed, packed RBCs on five or six occasions at 4 to 6 week intervals. The quantity of RBC suspension transfused varied from patient to patient depending on their body weight (10 cm^3/kg; Table 2). The rate of transfusion was 250 cm^3/h in

TABLE 2. Results of transfusion therapy in patients suffering from erythropoietic protoporphyria

Case No.	Transfusion (cm^3)		Tolerance to sunlight	PP level in red blood cells (μg/l)	Haematocrit	Haemoglobin (g/l)	Serum iron (μmol/l)
1.	6 × 750	Before therapy	20 min	6220	0·45	154	13·1
		After therapy	4–6 h	575	0·43	138	19·1
2.	6 × 700	Before therapy	15 min	6470	0·38	124	13·7
		After therapy	4–6 h	1920	0·36	126	18·1
3.	5 × 350	Before therapy	1 h	4450	0·36	118	8·4
		After therapy	All day	1050	0·40	130	17·2
4.	5 × 400	Before therapy	1–2 h	2700	0·40	130	9·1
		After therapy	All day	1920	0·40	130	11·1
5.	5 × 650	Before therapy	5 min	8670	0·48	162	12·9
		After therapy	30 min	1870	0·46	148	35·0
Normal values		Female		< 500	0·35–0·40	120–160	6·6–26·0
		Male		< 500	0·40–0·50	140–180	10·6–28·3

adults and 150 cm^3/h in children. Baseline laboratory tests were carried out immediately before the first treatment. The PP level in the RBCs, the haematocrit and the haemoglobin and serum iron levels were determined before every subsequent transfusion. In three cases the PP level in the RBCs was also determined 20 h after some transfusions. After the final treatment the liver function tests were again performed in all patients. The tolerance to sunlight was observed by the patients themselves. Of the tolerance to sunlight data in Table 2, those before treatment refer to the summer of 1981 and those after treatment to the summer of 1982. There was no appreciable meteorological difference between these 2 years in Hungary.

RESULTS

The results of the transfusions are summarized in Table 2. In every patient the completed course of transfusions reduced the PP level in the RBCs considerably; in spite of this, even after the treatment the level was higher than normal in all cases. The effects of the consecutive transfusions on the PP level in patients are depicted in Fig. 1. In each case the PP level on the day following the treatment was significantly lower; until the next transfusion it remained unchanged or rose very slightly. Six months after the final transfusion, the PP levels in the RBCs remained decreased.

By the end of the treatment the tolerance to sunlight was much improved in the first four patients, and a little improved in patient 5. The first four patients went on holiday for 2 weeks at Lake Balaton. The two children spent the whole day on the lakeside, while patients 1 and 2 were there for 2 to 3 hours in the morning or around midday without any complaints arising.

The haematocrit and haemoglobin levels and the results of the liver function tests remained

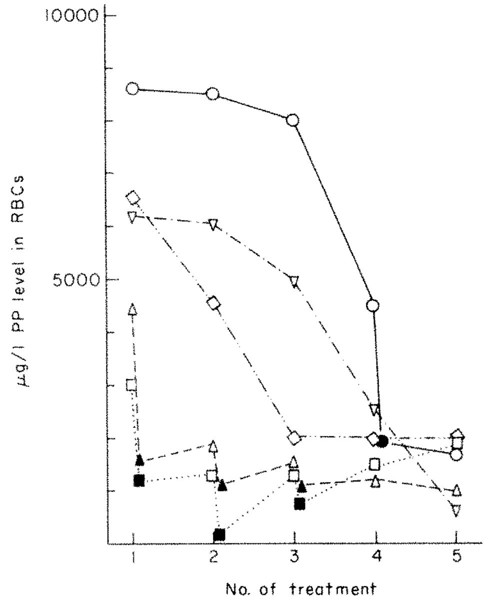

FIGURE 1. Effect of red blood cell transfusions on protoporphyrin levels in erythrocytes in patients with erythropoietic protoporphyria: patient 1 (▽), 2 (◇), 3 (△), 4 (□) and 5 (○). The empty symbols denote the results of examinations before the transfusion, and the shaded symbols the results of examinations on the day following the transfusion.

practically unchanged in the course of the treatment. In all cases the serum iron level was higher at the end of the treatment than before it, but only in patient 5 did it exceed the upper limit of the normal range.

DISCUSSION

Haematin infusion and erythrocyte transfusion have been employed successfully in several cases to treat certain forms of both hepatic and erythropoietic porphyrias (Bickers, 1981). In acute intermittent porphyria, haematin decreases the porphyrin precursor excretion and moderates the clinical symptoms (Bonkowsky *et al.*, 1977; Dhar *et al.*, 1975). Watson & Bosenmaier (1974) administered intravenous haematin to one patient with erythropoietic porphyria (Gunther's disease). This produced an apparent feedback repression of porphyrin biosynthesis, followed by a decline of porphyrin levels in the urine, plasma and erythrocytes. Erikson & Seip (1975) observed that a RBC transfusion caused a rapid dramatic fall in porphyrin excretion and a transient increase in light tolerance in a patient with Günther's disease.

Bechtel *et al.* (1981) transfused an 8-year-old EPP patient on eight occasions with washed, packed RBCs and observed a considerable enhancement in the tolerance to sunlight, lasting for more than 15 months; this reduction in photosensitivity was associated with a decline in the free erythrocyte PP levels. They postulated that the reduction in photosensitivity symptoms and PP levels in the patient after several transfusions may be due to suppression of the bone marrow synthesis of PP.

The origin of the PP found in the RBCs, plasma and stools of EPP patients is disputed. Because of the elevated levels of erythrocyte PP in this disease, the defect was originally thought to be erythropoietic. Various clinical observations and experimental evidence have led many investigators to question this original concept. The level of PP in the stools of a patient with EPP was found to be independent of that in the RBCs (Cripps & MacEachern, 1971). A number of studies have been carried out using isotopically labelled glycine and aminolaevulinic acid in EPP patients, with discordant findings. Abnormalities have been reported in hepatic (Nicholson *et al.*, 1973) and in erythropoietic (Schwartz *et al.*, 1971) tissues, as well as in both (Gray *et al.*, 1964; Scholnick, Marver & Schmid, 1971).

In the present study we observed that transfusions were followed in one day by a dramatic fall in PP levels in the erythrocytes, and these decreased levels were maintained for at least 6 weeks. The fact that RBC transfusions led to a rapid and marked depression of the PP in the RBCs seems to support the view that the liver plays no, or at most a minor role in the abnormal porphyrin production of patients suffering from EPP. The effect of the RBC transfusions in inhibiting porphyrin production may in part be a consequence of the depression of erythropoietin production, and in part a result of the release of an erythropoiesis-inhibiting factor i.e. an erythrocytic chalone (Erikson & Seip, 1975).

Piomelli, Lamola & Poh-Fitzpatrick (1971) demonstrated an extremely high concentration of PP accumulated in the RBCs in the later stages of maturation in the bone marrow. They observed that the PP disappeared rapidly as the cells entered the circulation. Most of the PP was lost after less than 3 days of circulation. We consider that the fast reduction of the PP level in the circulating RBCs after the packed cell transfusion may be due to the immediate suppression of erythropoiesis in the bone marrow.

In the children the PP levels in the RBCs were found to have decreased considerably after the first transfusion, whereas in the adults a similar decrease could be observed only after several transfusions (Fig. 1). Subsequently, there was hardly any further fall in the decreased PP level.

These observations indicate that the PP level in RBCs should be examined at regular intervals, and following the appreciable decrease the treatment may possibly be suspended.

It has recently become apparent that hepatic disease may be an important aspect of EPP. There is an increased incidence of cholelithiasis in patients with EPP, and several cases of fulminant hepatic failure due to cirrhosis with massive PP accumulation have been reported (DeLeo *et al.*, 1976; Mathews-Roth, 1980; Wells, Golitz & Bender, 1980).

The two common findings in patients with both EPP and the resulting fatal liver disease are the presence of extremely high PP levels in the RBCs and abnormal liver function test results (Bloomer, 1979; Mathews-Roth, 1980). Our findings lead us to the view that, in the case of a rapidly deteriorating hepatic function, the PP level can be decreased quickly and appreciably with packed cell transfusions. This presumably diminishes the development of hepatic insufficiency, for the liver damage is thought to be a consequence of PP accumulation in the hepatic tissues (DeLeo *et al.*, 1976; Cripps & Goldfarb, 1978; Wells *et al.*, 1980). However, there is no evidence at present indicating such a rapid decrease of the PP level in patients with protoporphyric liver disease.

Our results show that RBC transfusions produce a significant reduction not only in the PP level, but also in the photosensitivity. Long-term follow-up studies are necessary to establish how lasting is the enhanced sunlight tolerance attained with the repeated transfusions, and to elucidate the manner and the extent to which the treatment influences the initially impaired synthesis of porphyrin and haem and the iron metabolism. Transfusion therapy is recommended for the reduction of photosensitivity only after beta-carotene treatment has proved ineffective and after careful consideration of the risks involved in the treatment.

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Topical benzoyl peroxide increases the sebum excretion rate in patients with acne

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SUMMARY

Investigations on fifteen patients with acne showed that at the end of 1 and 2 months of treatment 5% benzoyl peroxide increased the sebum excretion rate by 22·5%. This rise in sebum excretion is probably due to the comedolytic activity of benzoyl peroxide which will influence the pooling of sebum in the upper reaches of the pilosebaceous duct.

There is controversy about the pharmacological effect of benzoyl peroxide. The bactericidal effect of benzoyl peroxide on *Propionibacterium acnes* and *Staphylococcus epidermidis* is unquestioned (Fulton, Farzad-Bakshadeh & Bradley, 1974; Cunliffe & Holland, 1981) but it has been shown to be sebosuppressive by two groups of investigators (Fanta & Jurecka, 1978; Gloor, Klump & Wirth, 1980) but not by Goldstein & Pochi (1981). Patients using benzoyl peroxide often complain of a dry skin, the result of a primary irritant dermatitis (Cunliffe *et al.*, 1981) but personal clinical observation suggests that the seborrhoea persists. The purpose of this paper was to help to resolve the debate as to whether or not benzoyl peroxide is sebosuppressive.

METHODS

Fifteen patients, ten females and five males, aged 18 to 22 years, with moderate acne were investigated. No patient had received any acne treatment for 8 weeks before the study. The treatment consisted of twice daily application of 5% benzoyl peroxide (Panoxyl® Gel 5). At the pre-treatment visit and after the first and second months of treatment the forehead sebum excretion rate was measured using the gravimetric technique (Cunliffe & Shuster, 1969).

RESULTS

The results are shown in Table 1. The treatment produced a significant elevation of the sebum excretion rate by up to 22·5% at both the first and second months of treatment ($P < 0{\cdot}001$).

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TABLE 1. The effect of benzoyl peroxide on sebum excretion rate ($\mu g\ cm^{-2}\ min^{-1}$)

Patient	Control SER	SER at 1 month	SER at 2 months
J.B.	1·28	1·81	1·82
E.C.	1·06	1·50	1·37
K.C.	1·56	2·71	1·39
J.C.	1·0	1·10	1·23
C.G.	0·74	1·02	1·04
S.H.	1·27	0·80	1·43
T.H.	0·77	0·74	0·92
I.N.	0·73	1·16	1·07
P.P.	1·68	2·08	2·05
S.R.	1·3	1·43	1·73
K.S.	1·21	1·30	1·44
G.T.	1·00	1·11	1·48
J.W.	1·17	1·35	0·86
A.W.	1·21	1·47	1·90
C.W.	0·73	0·85	0·77
	1·11±0·09	1·36±0·07	1·36±0·12

DISCUSSION

Even though treatment with benzoyl peroxide often produces a primary irritant dermatitis (Cunliffe *et al.*, 1981), the patient still complains of seborrhoea. Thus, it was a surprise when benzoyl peroxide was shown to have a sebosuppressive effect (van Fanta & Jurecka, 1978; Gloor *et al.*, 1980). These two groups of investigators used an indirect method of assessing sebum production. They used thymidine incorporation into the sebaceous gland and showed that benzoyl peroxide reduced thymidine uptake. The observation of a suppression of thymidine incorporation could be an artefact due to destruction of the thymidine by the highly oxidizing compound benzoyl peroxide. Goldstein & Pochi (1981) using more established methods for assessing SER, showed that benzoyl peroxide does not suppress sebum excretion. Our data confirms that benzoyl peroxide is not sebum suppressive. However, we ourselves were surprised to have demonstrated that the sebum excretion rate increased by 22·5% following treatment with benzoyl peroxide. We do not consider that benzoyl peroxide increases sebum production but suggest that benzoyl peroxide may influence sebum excretion, i.e. the delivery of sebum onto the surface of the skin. Benzoyl peroxide reduces non-inflamed lesions (Cunliffe, Dodman & Ead, 1978) and this will reduce the outflow resistance to sebum. It will also reduce the volume of sebum which can accumulate in the intercorneocyte crevices. Both these factors could explain the significant elevation of sebum excretion, an event which is probably of pharmacological benefit to the patient. A more efficient flow of sebum will reduce the likelihood of bacteria colonizing the pilosebaceous duct which is a prerequisite for the development of inflamed acne lesions. It is unlikely that the increased sebum excretion rate will be noticed by the patient especially in the presence of a primary irritant dermatitis (Cunliffe *et al.*, 1978; Hurwitz, 1979). Since concluding our laboratory study, we have asked 100 patients treated with benzoyl peroxide at the second month of treatment whether their skin was more or less greasy. Only four thought that their skin was more greasy, twenty believed the skin to be drier and the others were unable to comment adequately. However, it is fairly clear that benzoyl peroxide is not sebum suppressive. A small increase in sebum excretion rate following treatment with benzoyl peroxide adds further evidence to the comedolytic activity of this drug.

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Case Reports

Subcorneal pustular dermatosis with vesiculo-bullous eruption. Demonstration of subcorneal IgA deposits and a leukocyte chemotactic factor

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SUMMARY

We report an unusual case of subcorneal pustular dermatosis with widespread vesiculobullous lesions. Reduction of the oral prednisone dosage provoked a severe exacerbation, with erythroderma associated with massive pustule formation. This was effectively controlled by topical PUVA. Subsequent recurrence of skin lesions responded well to oral etretinate.

Investigation revealed intercellular IgA deposits at the upper epidermis of the paralesional skin, but no circulating antibodies were detected. Characterization of the chemotactic activity of the soluble components of the scales revealed the presence of a 12,000 molecular weight leukotactic factor resembling that found in psoriasis. Furthermore, the vesicle fluid showed only this chemotactic factor, indicating that it may be a major factor in the pathogenesis of subcorneal pustules.

Subcorneal pustular dermatosis (SPD) (Sneddon & Wilkinson, 1956) is a chronic disease characterized by the formation of sterile subcorneal pustules containing polymorphonuclear leukocytes (PMNs) on the abdomen and in the axillary and inguinal folds. The treatment of choice is dapsone or sulphapyridine. Its aetiology is unknown but recently a coexistent gammopathy, particularly involving IgA, has attracted special interest (Sneddon & Wilkinson, 1979).

We here present a case of SPD with prominent blister formation in whom we consistently demonstrated IgA deposits at the intercellular portion of the upper epidermis. Moreover the presence of a 12,000 molecular weight PMN chemotactic factor similar to that found in psoriatic scales (Tagami & Ofuji, 1977; Tagami *et al.*, 1982) was shown in the scales and vesicle fluid.

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CASE REPORT

A 50-year-old woman was admitted to hospital in July 1979 with a pruritic vesiculopustular skin eruption. Her history indicated that she first developed a slightly pruritic crusted rash on the right axilla at age 44. Over the past 6 years, during which she had been treated by several doctors without any definite diagnosis, the eruption had gradually spread to involve the scalp, trunk and limbs. A few weeks prior to admission, clear-cut vesicles began to develop among these lesions. No other members of her family had a similar skin eruption and her previous history was non-contributory.

Physical examination showed an afebrile obese woman (body weight 45 kg and height 136 cm). The skin surface showed irregular erythematous areas with numerous vesiculopustules, eroded areas and crusted areas (Fig. 1). They were particularly prominent on the axillae, inframammary regions, abdomen, lumbosacral region and extensor surface of the legs. The size of the vesiculopustular lesions ranged from 5 to 10 mm. They were flaccid or tense, some showing accumulation of pus in the lower half of the bullae. Nikolsky's sign was negative. The scalp was diffusely involved with pustule and crust formation, but the palms, soles and visible mucous membranes were spared. There was no lymph node enlargement.

Investigations

Laboratory examinations disclosed the following abnormal findings: WBC count, $9000/mm^3$ with 76% neutrophils, 12% eosinophils, 9% lymphocytes, 2% monocytes and 1% basophils; CH50 27 u (normal, 30–50); anti-streptolysin O titre, × 240 (normal less than 160); serum IgA, 470 mg/dl (normal 37–424). No monoclonal IgA was found.

Cultures from the vesicles grew no organisms. Tzanck smears prepared from the bullous lesions showed the presence of PMNs, several mononuclear cells and a few acantholytic cells whose surface was surrounded by these infiltrating cells, presenting a rosette formation.

A skin biopsy from a blister showed subcorneal bulla formation containing a small number of PMNs which were also migrating across the epidermis into the bulla (Fig. 2). The epidermal cells of the upper portion showed eosinophilic cytoplasm and there were a few acantholytic

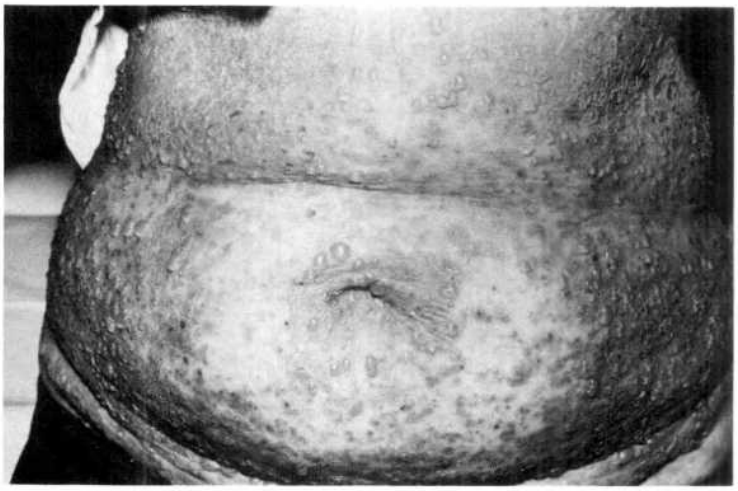

FIGURE 1. Numerous tense of flaccid vesiculo-bullous lesions on the abdomen.

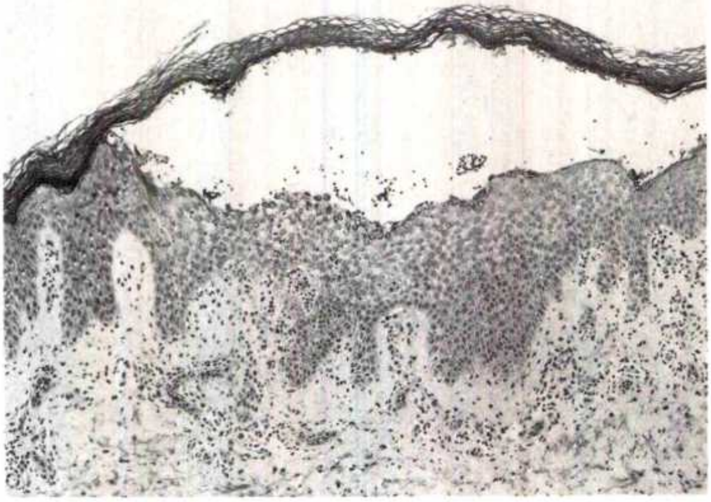

FIGURE 2. Histopathology of an early tense blister. Subcorneal bulla formation with a few acantholytic epidermal cells surrounded by polymorphonuclear leukocytes (H & E, × 100).

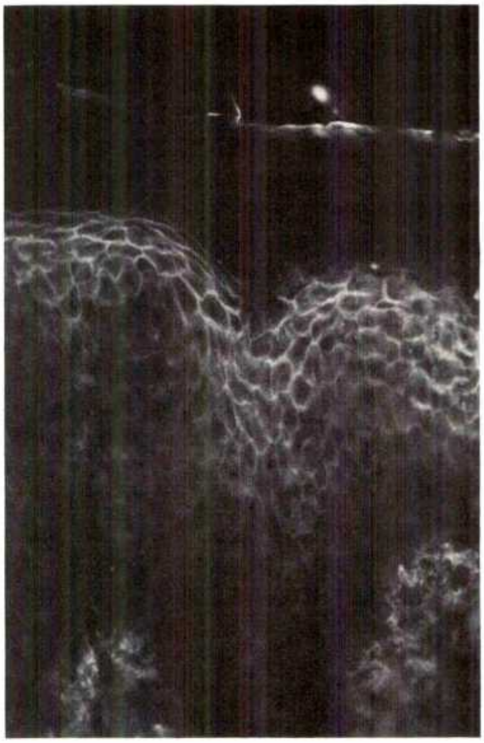

FIGURE 3. Intercellular deposition of IgA at the upper half of the epidermis.

eosinophilic epidermal cells surrounded by PMNs. Another biopsy taken from a fully developed pustular lesion showed a subcorneal pustule filled with PMNs and masses of eosinophilic degenerated epidermal cells. Pustule formation was also noted in the follicular epithelium.

Direct and indirect immunofluorescence tests were performed by the standard technique. Direct immunofluorescence of normal skin adjacent to lesions obtained on three different occasions always showed deposits of IgA at the intercellular portion of the upper epidermis (Fig. 3). In one specimen concurrent deposition of IgG, IgM, C1q and C3 was also demonstrated. Indirect immunofluorescence was consistently negative.

Polymorphonuclear (PMN) chemotaxis

For the characterization of PMN chemotactic factor involved in the formation of subcorneal pustules, the water-soluble components were extracted from a small amount of crust and scales taken from the lesional skin in phosphate-buffered saline (PBS; pH 7·4) supplemented with streptomycin (100 μg/ml) as reported previously (Tagami & Ofuji, 1977, 1978). The contents of tense blisters as well as the scale extract were applied to Sephadex G-75 and eluted in PBS, whose chemotactic activity was assayed using a modified Boyden chamber and guinea-pig peritoneal exudate PMNs as described by Tagami & Ofuji (1977).

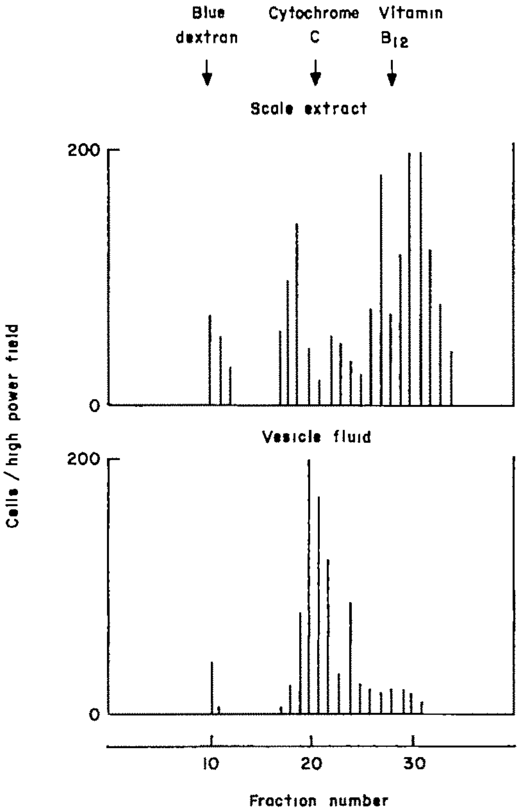

FIGURE 4. Characterization of leukocyte chemotactic activity in scale extract and in vesicle fluid by Sephadex G-75.

The elution pattern of the scale extract showed a multiphasic pattern of PMN chemotactic activity resembling that of psoriasis, with a definite peak with low protein contents eluting near the cytochrome C marker (molecular weight, 12,500 daltons) (Fig. 4). The elution pattern of the vesicle content revealed only the presence of this chemotactic peak in eluate near the cytochrome C marker (Fig. 4).

Course

At first we diagnosed this case as having pemphigus foliaceus on the basis of the clinical features with prominent bulla formation and the demonstration of subcorneal pustules with acantholytic cells. The immunofluorescence findings of deposits of immunoglobulins and complement at the intercellular portion of the epidermis and the good response to systemic steroid therapy made this initial diagnosis more likely (Fig. 5).

After one and a half months she developed a generalized erythema with many fine pustules which did not improve with an increase in the dosage of prednisone to 40 mg daily. At this point a diagnosis of subcorneal pustular dermatosis was made and dapsone 25 mg g.d.s. was added to oral prednisone, with remarkable improvement. However, 1 month later an attempt to reduce the dosages of prednisone and dapsone because of laboratory signs suggestive of haemolytic anaemia induced a severe systemic relapse of the skin lesions, with generalized erythema, massive pustulation with fever of 38 to 39°C, leukocytosis ranging from 10,000 to 18,000/mm^3 and lumbago. A trial of topical PUVA (0·3% 8-methoxypsoralen cream followed by UV-A

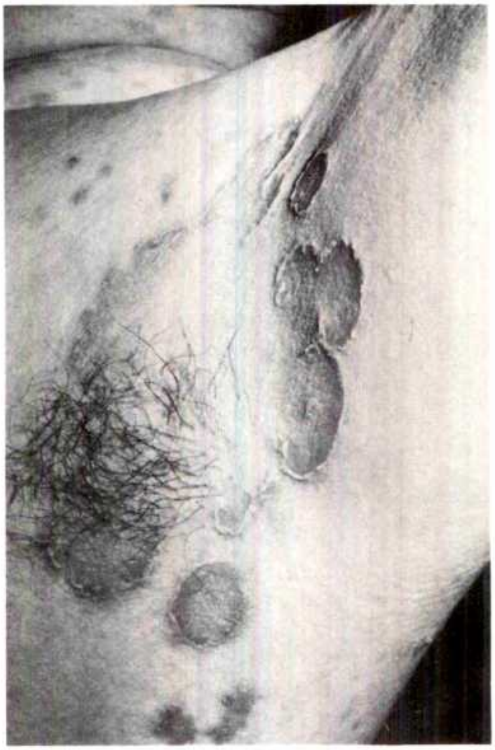

FIGURE 5. Pustules arranged in annular or circinate fashion in the axilla during the quiescent stage induced by prednisone steroid therapy.

irradiation 2 h later) proved to be effective on a part of the trunk and it was started all over the body. The skin lesions responded well to topical PUVA and 5 months after admission she was discharged from the hospital free from all medication.

Afterwards there was a slight recurrence of the skin lesions on the legs and buttocks but they were well controlled by oral administration of etretinate (Tigason®) 20 mg daily.

DISCUSSION

The patient presented with a chronic relapsing vesiculopustular eruption mainly involving the axillae, submammary and lumbosacral regions, which in a quiescent stage tended to coalesce forming circinate and gyrate patterns, similar to the typical cases of SPD reported by Sneddon & Wilkinson (1956, 1979). Histologically the fresh lesions showed a subcorneal bulla containing PMNs and a few eosinophilic degenerated acantholytic cells. Furthermore, dapsone appeared to be effective although evaluated only as an addition to oral prednisone.

Unusual points in this case are the histological extensive destruction of the epidermis and the widespread distribution of the lesions, including the scalp and lower legs. There was also prominent formation of vesicles and bullae.

Our initial diagnosis of unusual pemphigus foliaceus was militated against by the failure to demonstrate anti-epithelial intercellular IgG or IgM antibodies on repeated immunofluorescence tests, the distribution of the skin lesions sparing the face, and the poor response of the cutaneous lesions to oral prednisone. Furthermore, the acantholytic epidermal cells seemed to develop due to toxic degeneration caused by infiltrating PMNs which formed a dense cluster around the detached epidermal cells as shown by the Tzanck smears. Therefore we think that this case represents an atypical case of SPD with an extreme tendency to blister formation.

The consistent demonstration of intercellular deposits of IgA with or without complement in the upper epidermis of the paralesional normal skin is particularly interesting since the location of IgA deposits corresponds to the portion of the pustule formation. However, we could not demonstrate any circulating IgA intercellular antibodies. Gammopathy, particularly of IgA, has been occasionally reported in SPD (Wolff, 1971; Cream, Grimes & Roberts, 1977; Varigos, 1979; Ryatt, Dodan & Cotterill, 1981; Wallach *et al.*, 1982). Moreover, Sneddon & Wilkinson (1979) found IgA in the pustule attached to the PMNs in one case. Recently Wallach *et al.* (1982) found deposits of IgA kappa at the subcorneal zone of the perilesional non-involved epidermis; they further demonstrated circulating IgA kappa reacting with the same location of normal human epidermis. However, the pattern of deposition in their case was distinctly different from the present case since it occurred diffusely in the cytoplasm. Nevertheless, despite some variability in the behaviour of IgA in different cases we believe IgA deposition may be important in the pathogenesis of the skin lesions, especially in the induction of leukocyte chemotaxis and their clustering around the detached keratinocytes. Krogh & Tönder (1970) suggested an immunological mechanism may underlie the pustule formation in SPD on the basis of their demonstration of immune complexes in the lesional stratum corneum, and the lack of anti-stratum corneum antibody in the blister fluid. However, at present we do not have any explanation for the relationship between subcorneal IgA deposits and immune complex formation at the lesional horny layer.

Characterization of chemotactic activity of the lesional horny tissues using molecular sieve chromatography revealed the presence of a PMN chemotactic factor with a molecular weight near 12,000 daltons. It is of interest that a similar chemotactic factor, which is thought to be a C5-cleavage product, has been demonstrated in psoriasis, pustular psoriasis and pustulosis palmaris et plantaris (Tagami & Ofuji, 1977, 1978; Tagami *et al.*, 1982). All these dermatoses are characterized by the formation of microscopic or macroscopic sterile subcorneal pustules. Moreover, the vesicle content of the present case showed only this chemotactic factor, indicating that it may be a major factor inducing PMN migration into the subcorneal pustules.

The severe exacerbation of pustular lesions with generalized erythroderma on reduction of oral prednisone is similar to that which has been noted in pustular psoriasis (Baker & Ryan, 1968). Topical PUVA effectively abated the exacerbation in this case, and PUVA is reported to inactivate the chemotactic factor in psoriasis scales (Mizuno, Enami & Esaki, 1979) and complement-derived anaphylatoxin (C5a) (Mori *et al.*, 1980; Schröder & Christophers, 1982).

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Keratoderma and spastic paralysis

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SUMMARY

Punctate keratoderma and spastic paralysis occurred in three generations of a family. Several members had keratoderma of the palms and soles or spastic paralysis or both. The family history was consistent with autosomal-dominant inheritance. The age at onset and the rate of progression of symptoms were variable. The concurrence of these lesions can be interpreted to mean either that the keratoderma and the paraplegia are the pleiotropic effects of the same mutant gene or, less likely, that they are the manifestations of two different autosomal mutations segregating in this family. We are not aware of a similar syndrome having been previously reported.

Hereditary keratoderma is classified according to the mode of inheritance (dominant or recessive) and the pattern of involvement (focal, diffuse, or mutilating). Certain well-recognized clinical syndromes are associated with palmoplantar keratoderma, including mal de Meleda (with lesions on the dorsum of the hands and feet, legs, forearm, axilla, and trunk), Papillon–Lefèvre syndrome (with periodontal involvement, premature loss of teeth, and psoriasiform lesions on the knees), and Richner–Hanhart syndrome (associated with corneal dystrophy, osseous changes, and mental deficiency). Several miscellaneous syndromes also have been reported in association with hereditary keratoderma, usually in single families, and these syndromes probably are the pleiotropic effects of very rare or even 'private' mutations or the chance association of more than one genetic mutation. We describe a family of Norwegian origin which had focal punctate palmoplantar keratoderma associated with spastic paralysis mainly of the lower limbs. These appeared in three generations, with variable expression in some family members

CASE HISTORIES

A 51-year-old man (III-7 on the pedigree chart, Fig. 1) presented in April 1978 with 'callosities' that had been present on his palms since the age of 26 and on his soles since the age of 36. The

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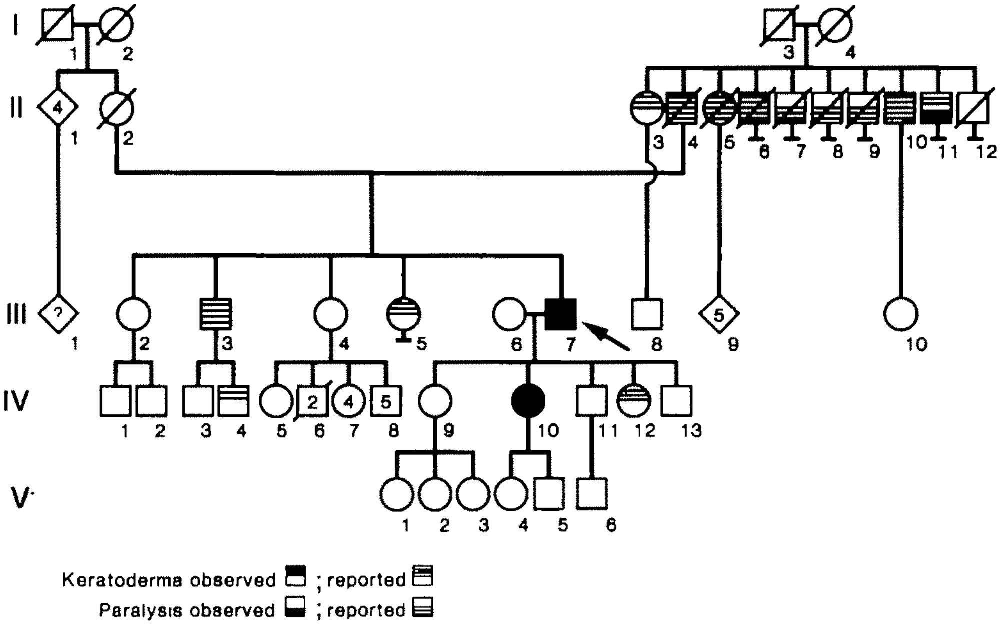

FIGURE 1. Pedigree of family with keratoderma and spastic paralysis

keratoderma extended progressively but was largely asymptomatic. The patient also complained of progressive weakness and stiffness of his legs. He had first noted that his gait was different from other children at the age of 8 years, when he began to 'wobble and stumble' when running, but he was not disabled until the age of 30 years, when he became weak in both legs. This weakness had progressed to the extent that he was unable to walk any distance without support, and he stumbled frequently. Since the age of 48 years, his arms also had been weak, but his general health otherwise was good.

He described several other members of his family who were similarly affected with keratoderma and lower limb weakness (Fig. 1). There was no history of keratoses or paralysis in his maternal lineage. His father (II-4) had callosities and weakness of the legs, and three paternal uncles (II-6, II-10, and II-11) and one paternal aunt (II-5) were reported also to have both callosities and weakness of the legs. Three paternal uncles (II-7, II-8, and II-9) were reported to have had weakness of the legs but no callosities, while one paternal aunt (II-3) was said to have callosities but no leg weakness. One paternal uncle (II-7) had a 'mental illness' at the age of 40 years, requiring hospitalization, but there was no other family history of mental disorder. As far as is known, all seven paternal first cousins (III-8, III-9, and III-10) were healthy, and the paternal grandparents (I-3 and I-4), who lived and died in southern Norway, were not known to have been affected. Of the patient's five siblings, two were reported to be affected. A brother (III-3) is said to have callosities on his feet and hands and progressive weakness of the legs since birth, to the degree that he was unable to walk without support after the age of 30 years. One of that brother's sons (nephew IV-4) had callosities on the hands since the age of 18 years. One of the patient's three sisters (III-5) is said to have callosities on her soles but no weakness of the legs.

Two of the patient's five children were reported to be affected. A daughter (IV-10), age 32 years, whom we examined (see below) has had palmar callosities since the age of 18 and weakness of the legs since the age of 29. Her daughter and son, ages 4 and 2 years, respectively—a granddaughter and a grandson (V-4 and V-5)—were not known to be affected. Another daughter (IV-12), age 28 years, was reported to have had small callosities on the soles since about the age of 24 years; her legs were not weak. None of the index patient's six grandchildren (V-1 through V-6) was known to be affected.

On examination, the index patient (III-7) had focal hyperkeratotic papulonodules scattered on the palms, most extensive on the lateral border about the hypothenar eminences, with shallow umbilications on their surfaces (Fig. 2A). The lesions on the palmar surface of the distal phalanges were more discrete and punctate, the umbilications giving a 'pitted' appearance to the skin in those areas. The lesions on the soles were larger and more hyperkeratotic, occurring

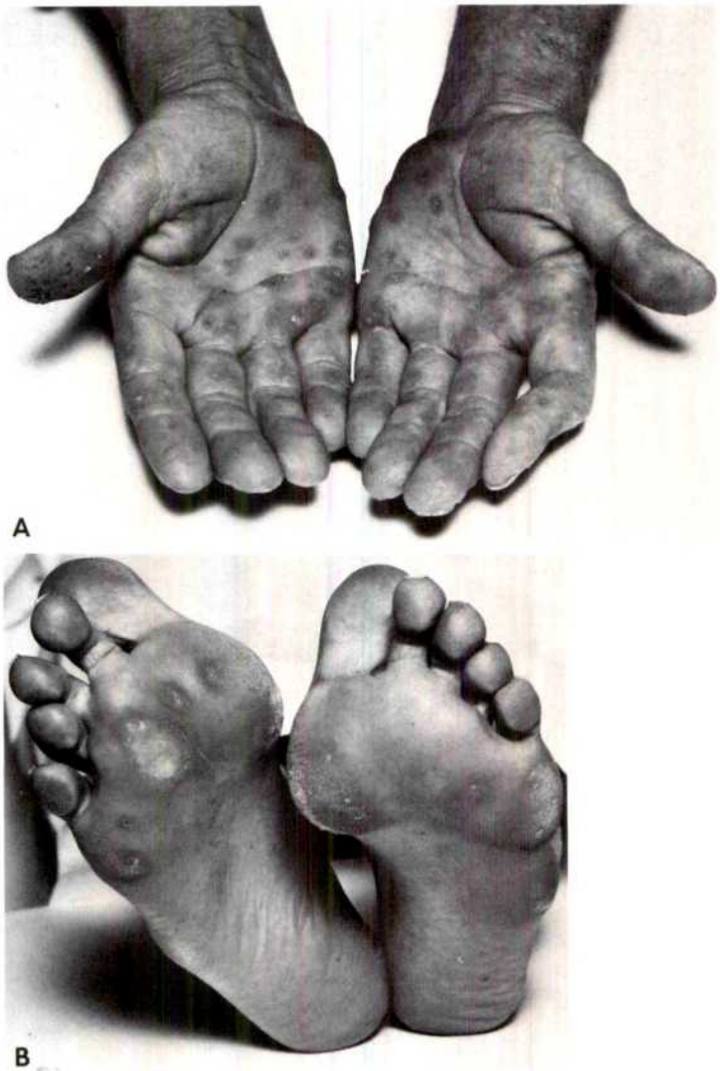

FIGURE 2. Focal hyperkeratotic papulonodules, with 'pitted' appearance most marked on distal phalanges of palms. (A) Palms. (B) Soles.

mainly at the metatarsal heads and other areas of pressure (Fig. 2B). Although less obvious than those on the palms, focal umbilicated areas were visible on the soles. Histological examination of a lesion from the palm showed pronounced orthokeratosis, acanthosis, papillomatosis (Fig. 3), and a regular undulation to the surface keratin. Special stains revealed no nerve, connective tissue, or sweat gland changes, and KOH and periodic acid-Schiff studies showed no evidence of fungal infection. A biopsy specimen from the sole revealed a similar histological pattern.

There was no evidence of other developmental anomalies, and the nails, teeth, and hair were normal. The patient was of normal intelligence, and there was no abnormality of the cranial nerves. Examination of the upper limbs revealed normal power, tone, coordination, and sensation, but reflexes in the biceps (+3), supinator (+3), and triceps (+1) were all increased. There was marked spasticity of the lower limbs. The patient walked on his toes, with his knees bent and a scissors gait, and required support. The Romberg sign was negative. There was moderate weakness of the quadriceps, extensors of the legs, and dorsiflexors of the feet. Cutaneous sensation (touch and pin prick), vibration sense, and positional sense were normal. Tendon reflexes at the knees and ankles were greatly increased (+3), and both plantar responses were extensor, consistent with spastic paraplegia. Electromyography showed evidence of a high peroneal neuropathy that was not at the head of the fibula, with both sural responses present but of low amplitude. Spinal roentgenograms were normal.

The index patient's daughter (IV-10), age 32 years, was subsequently examined. She had callosities that had developed on the hands at the age of 18 years and, shortly after, on the soles of her feet. At the age of 29 years while pregnant with her second child, she experienced low back

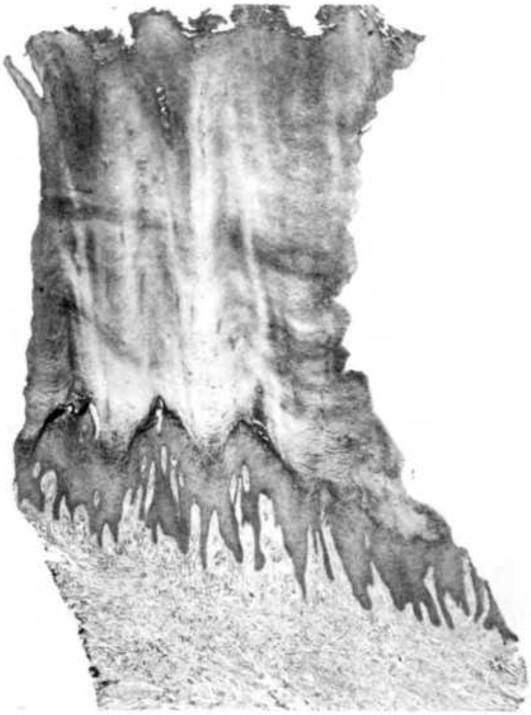

FIGURE 3. Biopsy of lesion from palm shows marked orthokeratosis, acanthosis, and regular papillomatosis, (Haematoxylin & Eosin, × 16).

pain and weakness of both legs. After an uncomplicated delivery of a normal infant, the back pain subsided, but the weakness persisted. It was most evident early in the mornings after rising and when hurrying. Otherwise, she seemed in good health.

Examination revealed cutaneous findings similar to those seen in her father, with numerous small keratoses over the proximal and middle phalanges of the second to the fifth fingers on both hands, some of which were umbilicated. The soles also showed keratotic plaques over the metatarsal heads, and there were numerous small punctate umbilicated keratoses on the instep. She had mild dorsal kyphosis of the spinal column with thoracolumbar scoliosis but no other skeletal abnormalities, and her hair, teeth, and nails were normal. She was of normal intelligence, and neurological examination of cranial nerves and upper limbs showed no abnormalities except for hyperreflexia (+3) in the upper limbs. There was bilateral ankle clonus and slight weakness of flexor muscles of the thigh, with a pronounced increase of all tendon reflexes (+4) in the lower limbs. There were extensor plantar responses bilaterally, and the gait was spastic. No other neurological abnormalities were noted.

Information was subsequently obtained from the Norwegian physician who attended the index patient's paternal uncle (II-11). He had non-progressive spastic paraparesis that developed at about the age of 62 years.There was no medical information available about the state of the uncle's palms and soles, but our patient was sure that this uncle suffered from a similar thickening of the skin.

DISCUSSION

Hereditary keratoderma is a rare cutaneous disease that usually appears initially in early childhood. Involvement is generally bilateral and symmetrical, but certain patterns of hyperkeratosis are recognized. The diffuse form (Thost–Unna type) is characterized by pronounced thickening of the skin of the palms and soles and sharp demarcation from the normal skin. The circumscribed type is characterized by focal aggregations of hyperkeratotic tissue on the palms and soles, usually most pronounced at areas of pressure. Mutilating keratoderma is associated with constricting fibrous bands about the digits, with progressive strangulation leading to bony changes and gross mutilation of the hands and feet (Wigley, 1929; Presley & Bonte, 1961). Our patients had circumscribed keratoderma with discrete yellow papulonodular lesions that had areas of central umbilication on the palms. They also had punctate keratotic and umbilicated papules on the palmar surface of the distal digits. These lesions in our cases are similar to those described as keratoderma punctatum (Sweitzer, 1923) or keratodermia maculosa disseminata palmaris et plantaris, which is known to be inherited in an autosomal-dominant fashion (Heierli-Forrer, 1959). In addition to well-defined clinical syndromes (for example, Richner–Hanhart, mal de Meleda, Papillon–Lefèvre), keratoderma has been described in association with many different defects (Table 1), usually seen in single families and probably representing unique or 'private' genetic mutations.

In 1947, Hanhart described four families that had keratoderma of the palms and soles associated with multiple lipomas in three families and dystrophic keratitis and imbecility in one. In 1964, Fred *et al.* described an autosomal-dominant form of palmoplantar keratoderma associated with leukoplakia. Hereditary palmoplantar keratoderma with oesophageal carcinoma has been described in two English families (Howel-Evans *et al.*, 1958; Clarke *et al.*, 1959). Acquired keratoderma also has been associated with internal malignancy, especially carcinoma of the bronchus (Powell & Mackey, 1981; Kerdel & MacDonald, 1982). Hypogonadism and infantilism were mentioned by Cockayne (1933) in association with hereditary keratoderma and

TABLE 1. Conditions associated with hereditary keratoderma

Multiple lipomas
Leukoplakia/oesophageal carcinoma
Infantilism/ovarian/thymus abnormalities
Skeletal deformities
Keratitis/cataract/optic atrophy
Deafness
Microcephaly/oligophrenia/mental retardation
Epilepsy
Charcot-Marie-Tooth disease
Friedreich's ataxia
Spastic paraplegia

mental deficiency, while skeletal abnormalities including osteopoikilosis, oxycephaly, asymmetry of the face and cranium, spina bifida, clubbed fingers, and hyperextensibility of the joints with abnormally long bones have been described by other authors (Franceschetti & Schnyder, 1960; Šalamon & Lazović, 1962). Periodontitis is well known in the Papillon–Lefèvre syndrome, and the association of progressive sensorineural deafness with diffuse palmoplantar keratoderma of the Thost–Unna type has recently been reviewed (Hatamochi *et al.*, 1982). Deafness also has been noted with mutilating keratoderma (Drummond, 1939). Corneal opacities, cataract, and optic atrophy have all been found in families with hereditary keratoderma of the palms and soles (Burns, 1915; Dimsdale, 1949; Žmegač & Sarajlić, 1965), and microcephaly with varying degrees of mental subnormality also has been noted in several families with this disorder (Brünauer, 1924; Hanhart, 1947; Touraine, 1954; Möslein, 1960; Šalamon, Grin, & Salčić, 1963). Other defects of the nervous system reported in association with keratoderma include Friedreich's ataxia (Haddad & Pipkin, 1948), Charcot-Marie-Tooth disease (Rabbiosi *et al.*, 1980) and epilepsy (Davies-Colley, 1879). Spastic paraplegia and mental retardation are features of the Sjogren–Larsson syndrome (Sjögren & Larsson, 1957), which was also initially described in Norway, but ichthyosiform erythroderma, which is a constant feature of this syndrome, was not present in our patients.

Familial spastic paraplegia has been described in association with other cutaneous changes. Abdallat *et al.* (1980) described three siblings of a Jordanian family with a distinctive syndrome of disordered skin and hair pigmentation (both hypopigmentation and hyperpigmentation) associated with progressive spastic paraparesis and peripheral neuropathy. Stewart, Tunell & Ehle (1981) reported a similar but dominantly inherited syndrome of spastic paraplegia, peroneal neuropathy, and crural hypopigmentation in a family in the United States. Neither family had evidence of keratoderma. It is interesting that our index patient, in addition to having evidence of spastic paraplegia, had electromyographic findings of peroneal neuropathy.

The family reported herein has a distinct heritable neurocutaneous syndrome characterized by a discrete focal palmoplantar keratoderma and slowly progressive spastic paralysis mainly of the lower limbs. The family history, indicating that this condition has occurred in three successive generations, with affected males and females and with male-to-male transmission, is consistent with autosomal-dominant inheritance, and medical examination of three persons, one in each generation, supports this. If it is true that nine of the ten members of the father's sibship were affected, then almost certainly one of the paternal grandparents (I-3 and I-4) must have been affected, but we have no reliable information about their medical histories.

The keratoderma and the paralysis may both be the pleiotropic effects of the same mutant autosomal gene, variably expressed in different members of this family, or less likely, each may be an expression of different autosomal mutations that, coincidently, are segregating in this family. Most of the reputedly affected paternal uncles and aunts are dead or inaccessible, and the information about them may not be reliable. Therefore it is not possible, by formal genetic analysis, to determine whether the keratoderma and the paralysis are expressions of the same gene mutation or of two closely linked gene mutations or of two non-linked mutations. It would be interesting to know whether a family member with only paraplegia might have a child with only keratoses or whether a family member with only keratoses might have a child with paraplegia. Should either occur, it will make the single-gene explanation more likely. Perhaps the future history of this family will help to elucidate the genetics of this distinctive neurocutaneous syndrome.

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Malignant pyoderma

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SUMMARY

Malignant pyoderma is a rare, chronic, destructive ulcerating skin disease of unknown cause that affects young adults, usually males. In the seven previously reported cases, the lesions were distributed mainly about the head and neck region, and some were associated with neurological disturbances. The disease is progressive but responds to high doses of systemically administered steroids. We here describe an additional case and a follow-up of a previously reported case that was managed successfully with corticosteroid and cyclophosphamide therapy.

Malignant pyoderma is a progressive, locally destructive ulcerating skin disease that mainly affects the head and neck region of young adults. Seven cases of this disease have been reported (Table 1). The group included five males and two females whose ages at onset ranged from 15 to 47 years, with a mean of 24.

Lesions may develop spontaneously or at the site of trauma and enlarge peripherally, with destruction of tissue. The head and neck region is always involved, and there is a predilection for the periauricular region. In early lesions, before ulcers develop, there may be multiple draining sinus tracts and surrounding erythema. Areas of ulceration show an elevated, irregular border with no undermining and no tendency to slough. The base is usually smooth, and there is often a discharge of purulent material. Established ulcers are not surrounded by erythema, and the borders are not necrotic as they are in pyoderma gangrenosum.

Histopathological examination of tissue from the edge of the ulcer is non-specific, showing features of abscess formation. Material cultured from patients does not grow significant pathogenic organisms. Of the previously described patients, three have had transient cranial nerve palsies and one had transient unilateral sensory motor loss, motor dysfunction and mental confusion. The disease pursues a relentless course without remission, and two patients have died of complications.

We report another case of malignant pyoderma and a follow-up of a previously reported case.

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TABLE 1 Cases of malignant pyoderma reported to date

Case	Authors	Sex and age at onset (yr)	Location of lesions	Neurological disturbances	Treatment and response	Course
1	Present report	M, 19	Periauricular, face, upper trunk	None	Antibiotics, none; sulphapyridine, none; sulphones, none; intralesional steroids, none; cyclophosphamide, (?); thalidomide, (?); prednisone, positive.	Stable with prednisone and thalidomide therapy
2	Salassa *et al.** (1981)	M, 15	Periauricular, face, neck, upper trunk	Transient unilateral sensory motor loss and confusion	Surgery, none; irradiation, none; sulphones, none; clofazimine, (?); cyclophosphamide, positive; prednisone, positive	Healed and well, no therapy
3	Perry *et al.* (1968)	M, 17	Periauricular, face, neck, upper trunk	Transient cranial nerve palsy (VII) and footdrop	Prednisone, positive	Died of malignant hypertension 3 yr after onset of skin lesions
4	Perry *et al.* (1968)	M, 44	Periauricular, face, neck, right hand and forearm	None	Prednisone, positive	Healed and well
5	Perry *et al.* (1968)	F, 18	Periauricular, face, neck, upper trunk	Cranial nerve palsy (III, IV, VI, and VII), sensory loss (V_1 and V_2)	Prednisone, positive	Died of disease (granulomatous vasculitis? or Wegener's granulomatosis) 5 yr after onset of skin lesions
6	Anderson (1978)	F, 47	Cheek, forehead	None	Antibiotics, none; surgery, none; sulphones, none; local injection of corticosteroids, positive	Healed and well
7	Walther & Poh-Fitzpatrick (1978)	M, 22	Periauricular, face, neck, upper trunk, thighs (few)	None	Surgery, none; irradiation, none; prednisone, positive	Healed and well
8	Salassa *et al.* (1981)	M, 32	Periauricular, neck, upper trunk, face	Transient cranial nerve palsy (VII)	Surgery, none; irradiation, none; sulphones, none; azathioprine, none; clofazimine, (?) positive; leukopheresis, none; prednisone, positive	Healed, requiring continuous prednisone treatment

* Case 2 of present report

CASE REPORTS

Case 1

This 21-year-old male patient was initially seen by us in May 1981, with multiple, small discrete ulcers (0·5 to 1·5 cm) scattered over his back, several larger ulcers on both cheeks and neck (Fig. 1a, b), and an irregular, ovoid ulcer (5·0 by 3·0 cm) in the right maxillary area. The edges of the ulcer were elevated, and the base was covered with purulent material. The border was not undermined, and there was no surrounding zone of erythema. Physical examination was otherwise normal, and there were no neurological signs or symptoms. He had a 2-year history of recurrent ulcers on the cheeks and periauricular areas, which had been unresponsive to systemic antibiotic therapy and only partially suppressed by prednisone, in doses ranging from 30 to 80 mg/day, and sulphapyridine, 1 g/day. Results of extensive investigations were normal. They included haematological indices, blood chemistry studies, sedimentation rate, urinalysis, level of glucose-6-phosphate dehydrogenase, serum protein electrophoresis, cryoglobulins, antinuclear antibody, stools for occult blood, chest roentgenogram, bowel roentgenograms, chest fluoroscopy, liver and spleen scan, electrocardiogram, fluorescent treponemal antibody test, and thyroid function studies. Blood cultures were negative. Skin biopsy specimens from ulcers on the left side of the cheek and left side of the neck showed non-specific abscess formation.

Initially, he was treated with intralesional triamcinolone and tetracyclines systemically, together with prednisone, 40 mg orally per day, and Vleminckx hot packs to the lesions three times a day. After temporary improvement with this therapy, the lesions began to enlarge. The dose of prednisone was increased to 20 mg three times a day and sulphasalazine 1 g three times a day was added, with stabilization of the lesions and partial healing. In December 1981, after an episode of physical stress, he had pronounced exacerbation of the disease with extension of his facial ulcers (Fig. 1c), and new lesions developed on the anterior aspect of his chest. Cyclophosphamide, 100 mg/day, was added to the regimen for 4 months, but his lesions continued to enlarge. In May 1982, the prednisone dose was increased to 30 mg three times a day with rapid re-epithelialization of his ulcers, and within 4 weeks 60% healing had been achieved. The prednisone dose was reduced to 20 mg three times a day, and thalidomide, 100 mg twice a day, was added. With this regimen, the lesions have continued to heal.

Case 2

Details of this case have been reported elsewhere (Salassa, Winkelmann & McDonald, 1981). In 1977, a discharging nodulopustular lesion developed anterior to the left ear of this 15-year-old boy which was unresponsive to systemically administered antibiotics and recurred after surgical excision. Intralesional triamcinolone, irradiation, dapsone, and prednisone (30 mg/day) were all ineffective, and during the next 2 years, the patient continued to have multiple progressive, draining ulcers of his head, neck and upper part of his back. He also had three episodes of transient left-sided dysaesthesiae, weakness, and clumsiness that lasted from 3 days to 1 month, and these were associated with reduced memory on one occasion. Extensive neurological investigations, including a computed tomographic scan, lumbar puncture, and visual evoked responses, failed to reveal any specific cause, and a presumptive diagnosis of post-viral encephalitis was made. At observation in November 1979, he had multiple large ulcers on the cheeks, chin, and left auricular region, with well-defined borders and no peripheral erythema (Fig. 2), and multiple smaller lesions scattered on the upper back and posterior part of his neck. Physical examination was otherwise normal. Extensive investigations revealed no abnormali-

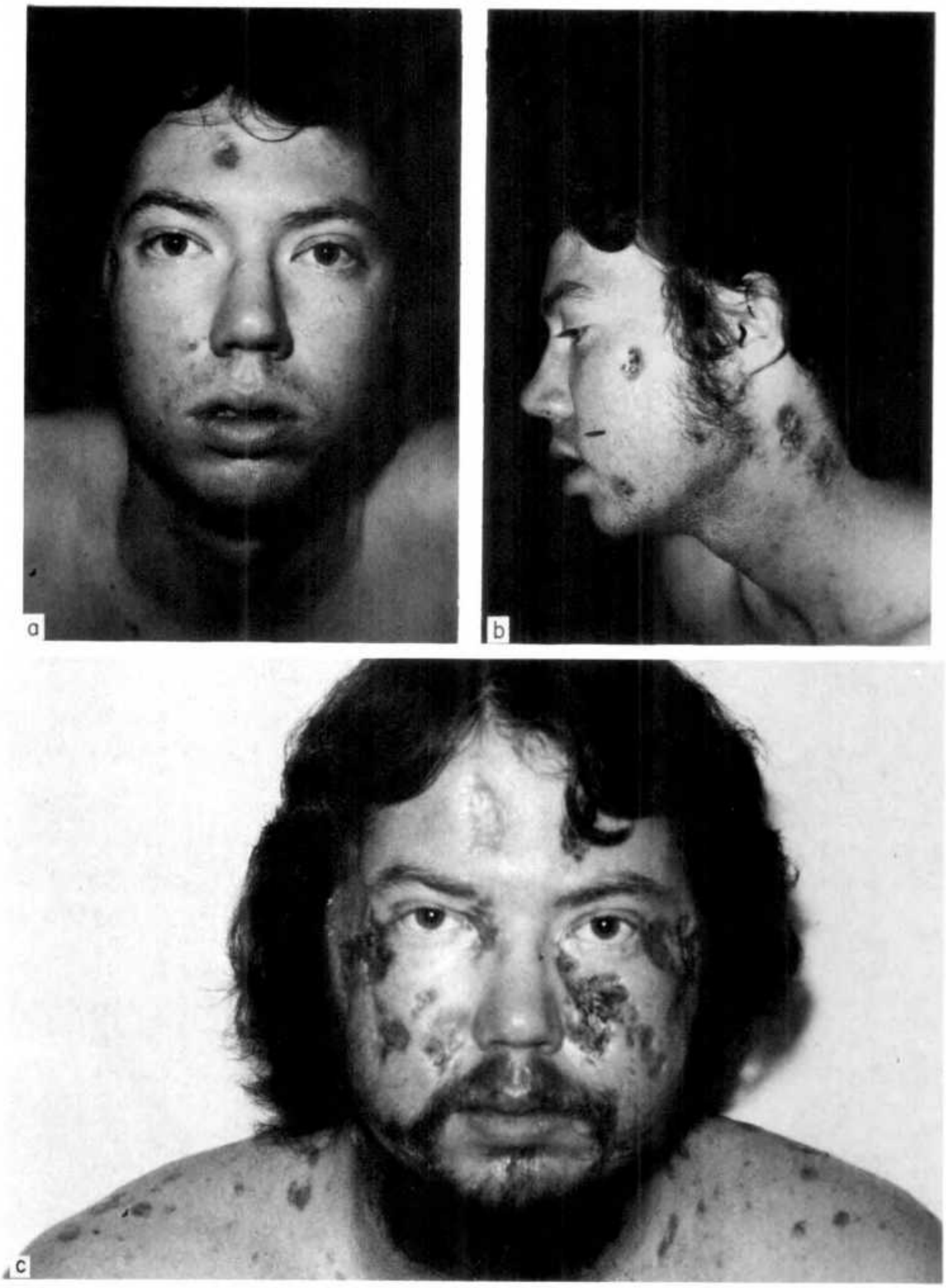

FIGURE 1. (case 1). (a) and (b) At initial presentation, with well-defined ulceration of the neck and maxillary areas. (c) One year later, with Cushingoid appearance and multiple enlarging ulcers.

ties. The prednisone dose was increased to 15 mg every 6 hours, and sulphapyridine 2 g daily was added. On this regimen, the lesions began to heal.

Two subsequent attempts, 3 and 6 months later, to decrease the dose of prednisone resulted in pronounced flaring of the disease. Clofazimine, 100 mg three times a day, was added for 3 weeks,

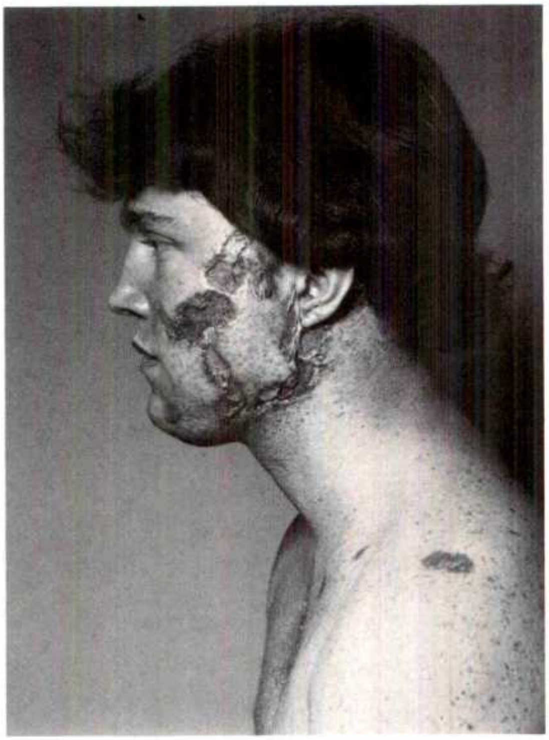

FIGURE 2 (case 2). Large ulcers in periauricular distribution.

without clear benefit. In June 1980, therapy was commenced with cyclophosphamide, 200 mg daily, in addition to prednisone, 60 mg daily, and the patient had a rapid, continuous and complete healing of all skin lesions. The dose of prednisone was reduced gradually and was discontinued in October 1980. Cyclophosphamide was continued at 200 mg daily until January 1981, at which time the dose was decreased to 400 mg/week. In October 1981, it was reduced to 200 mg weekly and finally discontinued in March 1982. In January 1981, transfer factor was given once per week, being reduced to once per month subsequently. The patient discontinued all oral medications in March 1982 and has remained well without recurrence of the lesions.

DISCUSSION

In considering the differential diagnosis of any progressive ulcerating disease of unknown cause, pyoderma gangrenosum must be seriously considered; a case of probable malignant pyoderma has been reported as 'pyoderma gangrenosum in an atypical distribution' (Walther & Poh-Fitzpatrick, 1978). We believe, however, that the disease pattern in malignant pyoderma is distinctive. The diagnosis of pyoderma gangrenosum is based on the clinical appearance of irregular ulcers with undermined bluish borders surrounded by an active zone of erythema (Brunsting, Goeckerman & O'Leary, 1930), whereas these features are not seen in malignant pyoderma. The lesions of pyoderma gangrenosum are typically distributed about the buttocks and lower legs, and often there is some response of the slowly enlarging lesions to local therapy. The rapidly enlarging ulcers of malignant pyoderma have an abrupt onset, occur on the head and neck region, and usually expand relentlessly in spite of local measures. The average age at onset of patients with pyoderma gangrenosum is 40 years (Powell *et al.*, 1983b), much older than that of patients with malignant pyoderma, and associated systemic disease is seen in most patients with pyoderma gangrenosum (Powell *et al.*, 1983a). None of the reported patients with malignant pyoderma have had other systemic diseases, although one patient subsequently died of a granulomatous vasculitis suggestive of Wegener's granulomatosis. Malignant pyoderma is similar to pyoderma gangrenosum, however, in that initial lesions may occur at the site of trauma (pathergy), and there is a consistent response of both diseases to high-dose systemic steroids.

Hidano & Watanabe (1981) described a 15-year-old boy with Takayasu's arteritis in whom a large pre-auricular ulcer developed and whose course was complicated by difficulty in concentration and paraesthesiae. In their review of the Japanese literature, they found that 30% of patients with pyoderma gangrenosum-like lesions had associated disorders of the cardiovascular system and that most of them had Takayasu's arteritis. Patients with arteritis had the onset of ulceration at a much earlier age (20 to 29 years) than did patients without arteritis, and many had ulcers about the head and neck region. The similarity to our patients who had malignant pyoderma is striking, but we have been unable to document large-vessel disease in our patients either clinically or, in case 1, by fluoroscopy.

Infection must be considered in the differential diagnosis of malignant pyoderma, but pathogenic organisms have not been isolated and prolonged courses of several antibiotics have not cleared lesions of malignant pyoderma (Anderson, 1978). Other conditions to consider include halogenodermas, factitial lesions, and vasculitis. The fact that one of the previously reported patients developed a granulomatous vasculitis is suggestive in this regard, and patients with Wegener's granulomatosis are known to develop skin ulcers that resemble those of pyoderma gangrenosum (Reed *et al.*, 1963; Hu, O'Loughlin & Winkelmann, 1977). However, we have been unable to demonstrate vasculitis in the skin of any of our patients in spite of numerous biopsy specimens being taken. The presence of neurological disturbances in 50% of patients would be consistent with systemic vasculitis, but again extensive neurological investigation in case 2 failed to reveal a distinct vascular pathogenesis.

Both of our cases and the previously reported cases emphasize the consistent response of the lesions to large amounts of steroids administered systemically in divided doses. With inadequate levels, there is partial suppression of the disease without complete healing, and side-effects of steroids ensue without significant benefit to the patient. Reduction of steroid dosage, however, is usually associated with flaring of the disease. In an effort to overcome this problem, sulphones, sulphapyridine, azathioprine, and clofazimine have been used in combination with systemically administered steroids, but without clear benefit in our patients (Table 1). Cyclophosphamide, in combination with prednisone, arrested the ulceration and resulted in complete remission of all ulcers in case 2. This regimen was of no benefit in case 1, although the dosages of both prednisone and cyclophosphamide were probably inadequate and the duration of treatment was insufficient. Transfer factor, given in case 2, is unlikely to have had significant effect, as the healing process was well established at the time it was given.

Thalidomide has been of benefit in some cases of pyoderma gangrenosum (Venencie & Saurat, 1982), and the initial response in case 1 is encouraging. Our current recommendation for the treatment of malignant pyoderma is high-dose corticosteroid and cyclophosphamide therapy to induce complete remission, as in treatment for granulomatous vasculitis. Further studies should provide more insight into the pathogenesis of this aggressive, pyodermatous ulcerating disease.

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Pigmented lesions of the tongue in heroin addicts—fixed drug eruption

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SUMMARY

Pigmentation of the tongue is an uncommon finding. We describe a case in which it was due to a fixed drug eruption following the inhalation of heroin pyrolysate and methaqualone vapours.

In all races the tongue is usually not pigmented. However, in the more heavily pigmented people hypermelanosis of the fungiform and less frequently of the filiform papillae occurs (Monash, 1932; Dummett, 1946; Kaplan, 1961; Koplon & Hurley, 1967; Holzwanger, Rudolph & Heaton, 1974). Macular pigmentation of the tongue seems to be related exclusively to a pathological condition.

Nine out of forty-seven patients (thirty-four Negroids and thirteen Caucasians) with a spongiform leucoencephalopathy after inhaling 'heroin' pyrolysate and methaqualone vapours had pigmentation of the tongue (Wolters *et al.*, 1982). All of them were Negroids, whereas none of the Caucasian patients had pigmentation of the tongue. We also noted pigmentation of the tongue in six Negroid addicts using heroin in a similar way and who attended the hospital for other reasons.

CASE REPORT

At the beginning of 1981, there was an epidemic in Holland of leucoencephalopathy of obscure aetiology among drug abusers inhaling the vapours of heated heroin. The total number of victims was forty-three, of whom eleven died (Wolters *et al.*, 1982). A 32-year-old Negroid man was admitted for observation to the Department of Neurology (Academic Hospital, University of Amsterdam) in February 1981. The patient had been using heroin since 1972 and he was smoking the heroin often in combination with cocaine, methaqualone and methadone. In January 1981 he noticed disturbance of speech and walking. He had never paid attention to the

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pigmentation in the mouth, but had ulceration of the tongue on several occasions that coincided more or less with smoking drugs.

Physical examination revealed a dark macular pigmentation and localized ulceration on the dorsum of the tongue (Fig. 1a). The ulceration healed quickly leaving depigmented and hyperpigmented macules (Fig. 1b). In the course of several weeks the pigmentation disappeared gradually. There were cerebellar signs with disturbed speech and ataxia. Cranial CT-scan showed low densities in the cerebral and cerebellar white matter. In the following weeks the patient showed no further deterioration and he later showed a partial improvement. After discharge from hospital the patient swallowed tablets of methaqualone which did not result in a recurrence of the pigmentation.

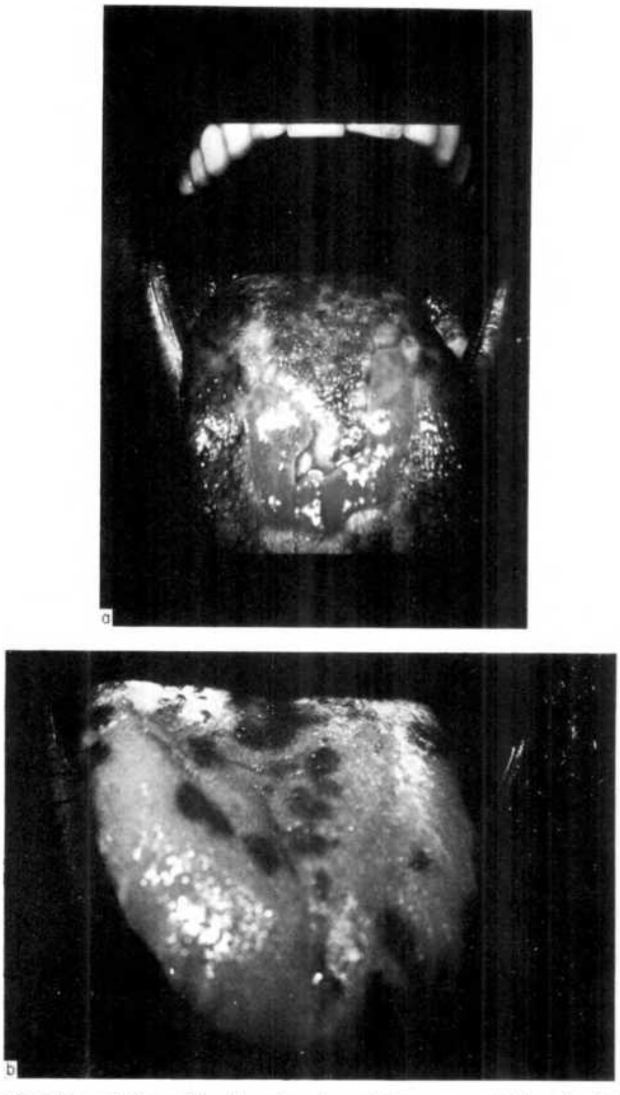

FIGURE 1. (a) Macular pigmentation of the dorsal surface of the tongue with localized ulceration. (b) After healing of the ulceration only macular pigmentation remained.

METHODS

Punch biopsies were taken from the eroded lesions and from the pigmented margins as well as from the normal dorsal tongue mucosa from six of the patients.

Light microscopy

The specimens were fixed in neutral buffered formalin, dehydrated in alcohol and xylol and embedded in paraffin wax. Five-micrometer sections were cut and stained with haematoxylin and eosin.

Electron microscopy

The samples were fixed for at least 2 days in a cacodylate buffered 1 : 1 mixture of glutaraldehyde and formaldehyde according to Karnovsky at 4°C, stained for 1 h in 1% uranyl acetate at room temperature, dehydrated in graded series of ethanol and embedded in Epon 812. Thin sections were cut perpendicular to the free surface of the skin. These sections were stained with uranyl acetate and lead, and studied with an electron microscope.

RESULTS

In our histological study we focused our attention on the non-specialized mucosa of the tongue, thereby excluding the description of the filiform, fungiform and vallate papillae.

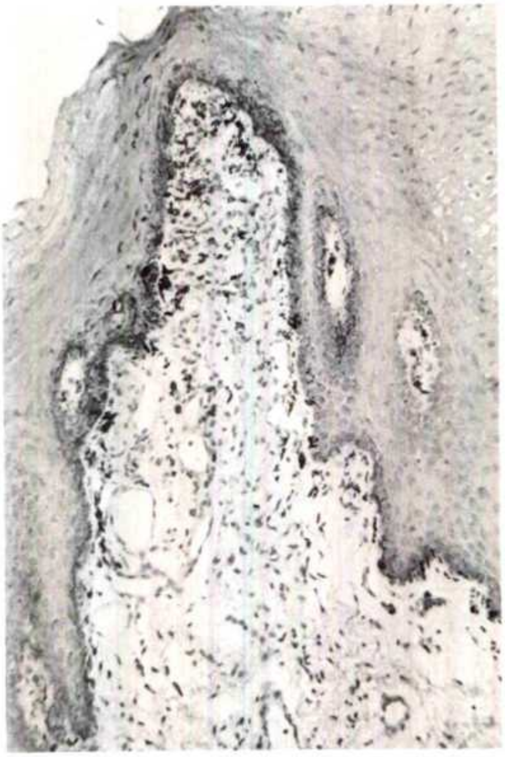

FIGURE 2. Fontana staining demonstrating increased melanizing pigmentation of the basal layer of the epidermis and of the dermis, (× 112).

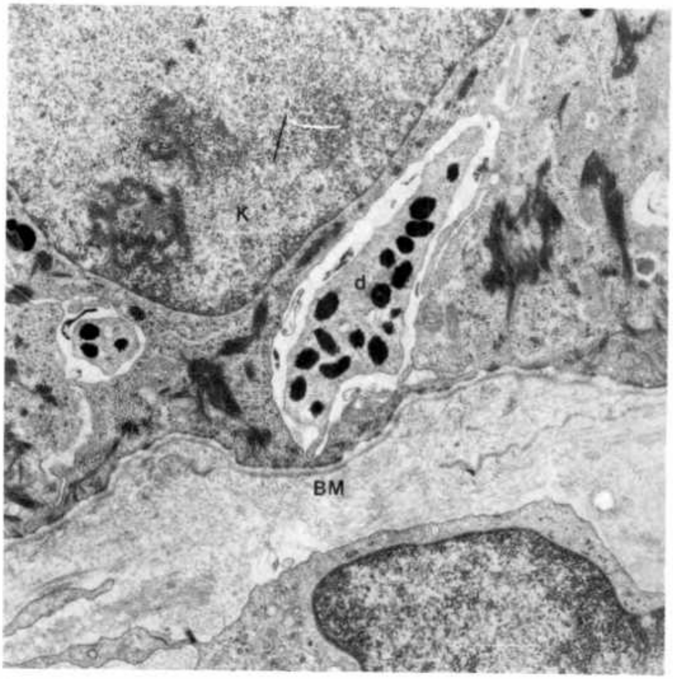

FIGURE 3. Dendrite of a melanocyte. The transfer of melanosomes to the keratinocyte is moderate. K = keratinocyte; d = dendrite; BM = basement membrane (× 11,300).

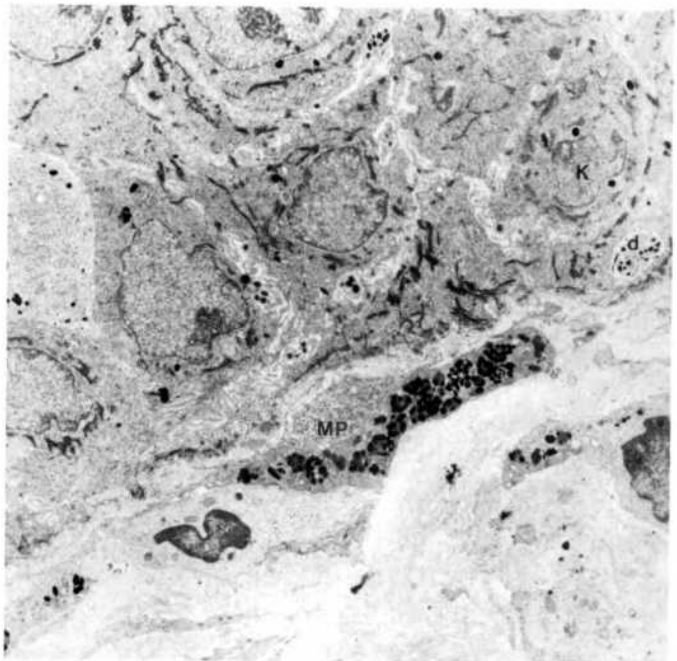

FIGURE 4. Macrophage with lysosomal structures containing (partly degraded) melanosomes. K = keratinocyte; MP = macrophage; d = dendrite (× 3,380).

The lesional (pigmented) mucosa was acantholytic with an extensive parakeratosis of the upper spinous and horny layer. There was a spongiosis and focal degeneration of the basal cells. The papillary dermis contained many pigment-laden macrophages (Fig. 2). The blood vessels in the dermis were widened and surrounded by a dense mononuclear infiltrate.

Electron microscopy of the lesional mucosa showed many active melanocytes that were arranged regularly in the basal layer and that were sometimes in apposition with white blood cells. Many dendritic processes were seen to be full of melanosomes (Fig. 3). In the keratinocytes a moderate number of melanosomes were present. There were many melanophages in the papillary dermis (Fig. 4).

DISCUSSION

Pigmentation of the tongue has been described in a number of pathological conditions (Table 1). The clinical development and histological appearance of the lesions on the dorsum of the tongue in our patients was compatible with a fixed drug eruption. According to the histories of our patients with pigmented macules of the tongue, the heroin was smoked in combination with methaqualone. Negroid patients without the tongue lesions denied taking methaqualone. We do not know whether heroin pyrolysate and/or methaqualone vapour was responsible for the fixed drug eruption.

Fixed eruptions have been reported for morphine, opium and codeine (Derbes, 1964) but not for heroin. Methaqualone ingested orally has been said to produce pigment changes in the tongue (Kessel *et al.*, 1967). However, the illicit retaking of methaqualone by one of our patients did not give rise to a reappearance of the pigmentation. The classical provocation test for a fixed drug eruption was not carried out in our patients for ethical reasons. It was noted that during the hospital admission of our patients the pigmented lesions faded gradually.

It is our experience that the macular pigmentation of the tongue is seen only in patients who smoke the heroin with methaqualone and inhale the vapours. No pigmentation was seen in addicts who injected their heroin or who took oral methaqualone.

TABLE 1. Pigmentation of the tongue in different diseases

Disease	Reference
Haemochromatosis	Burkett, 1971
Pernicious anaemia	Burkett, 1971
Scleroderma	Burkett, 1971
Peutz-Jeghers	Holzwanger *et al.*, 1974
Addison's disease	Kitamura & Mishima, 1957
Bismuth and arsenic deposits	Holzwanger *et al.*, 1974
Amalgam tattoo	Holzwanger *et al.*, 1974
Lichen planus oris	Laskaris *et al.*, 1981
Naevus of Ota and Ito	Mishima & Mevorak, 1961
Cancer chemotherapy	Krutchik & Ruzdar, 1979
Pigmented naevi, lentigo, melanoma	McCarthy & Shklar, 1964
Fixed drug eruption	Tagami, 1973

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Herpes simplex infections in pemphigus: an indication for urgent viral studies and specific antiviral therapy

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SUMMARY

Four cases of herpes simplex virus infection in exacerbations of pemphigus are described. Three of these patients had severe generalized infection requiring systemic anti-viral therapy. The presenting features, methods for rapid viral diagnosis and appropriate treatment are discussed.

Patients with vesiculo-bullous skin conditions are always at risk of infection by the herpes simplex virus (HSV). Nowadays virus laboratories can offer rapid diagnostic methods to confirm herpetic infection, and potent anti-viral agents have become available so that early specific treatment is possible. This is particularly important in patients with extensive skin involvement requiring high doses of corticosteroid and other immunosuppressive therapy (Keane *et al.*, 1976).

VIROLOGICAL METHODS

Swabs were taken from areas suspected of having viral infection; that is with painful small vesicles or ulcers. Unroofed vesicle or ulcer bases were swabbed firmly, the swab being placed in a virus transport medium. Specimens were inoculated into fresh monolayers of MRC-5 (human fibroblast) cultures and isolates were identified as herpes simplex virus by a standard neutralization test. The isolates were typed by viable membrane immunofluorescence, using type-specific antisera, based on the method of Nahmias *et al.* 1969).

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CASE REPORTS

Case 1 (male, 70 years) had a 55-year history of chronic benign familial pemphigus, controlled with betamethasone valerate, Betnesol® 1 mg daily and topical corticosteroids. He remained well until 10 May 1978 when he presented with sudden worsening of the rash with small blisters, which burst and were extremely painful. On examination, he had extensive tender erosions in the axillae, antecubital fossae, perineum and medial aspect of the thighs. Swabs for viral culture and a Tzanck smear for Giemsa stain were taken from the ulcers in the perianal area. He was treated with erythromycin 250 mg qds and potassium permanganate soaks. Herpes simplex virus type I was isolated from the swab, and Giemsa stain revealed giant cells in the Tzanck smear. He improved spontaneously.

Case 2 (female, 66 years) had a 5-month history of pemphigus foliaceus, requiring high doses of prednisone (60 mg daily) and azathioprine (100 mg daily) to control her disease. On 2 January 1979 she presented with considerable worsening of her blisters: they had become extremely painful and there was a general redness of the skin. This patient had a past history of cold sores. Swabs for virus culture were taken daily from painful ulcers, and herpes simplex virus type 1 was isolated from these and from a high vaginal swab. On 11 January 1979 she was started on vidarabine 200 mg IV every 8 hours (10 mg/kg/24 h) and this was continued for 5 days. By 19 January 1979 the skin was dry and clear of blisters.

Case 3 (male, 26 years) had a 1-year history of benign familial pemphigus. His moderate disease affected the flexures and was controlled with topical corticosteroids. He presented on 18 March 1980 with a 48-hour exacerbation of the eruption in his groin, scrotum and axillae, with very severe local pain. Small grouped blisters suggestive of a herpetic infection were noted at the edge of the pemphigus erosions and swabs from these were sent for viral culture and bacteriology. Herpes simplex virus type I was isolated. Treatment with topical 40% idoxuridine in DMSO for 24 hours gave no relief of his pain. Intravenous acyclovir therapy was begun, using 5 mg/kg body weight IV 8 hourly. After 5 days fresh herpetic lesions were still appearing so the dose of acyclovir was raised to 10 mg/kg 8 hourly for a further 3 days. No further herpes lesions developed and the pemphigus subsided over the next 2 days. This patient had no previous history of herpes infection, and his complement-fixing antibody titre to HSV rose from a titre of 1:10 on day 6 to 1:320 within 10 days.

Case 4 (male, 60 years) had a 15-month history of pemphigus foliaceus. His disease was difficult to control and required prednisone 20 mg qds and azathioprine 50 mg tds. He presented on 5 August 1981 with a 1-week history of conjunctivitis and an exacerbation of his pemphigus involving the cheek, chest and arm. The skin was very painful. Swabs from the eye, cheek, ear and chest were taken for virus culture, and herpes simplex virus type I isolated from each. Ophthalmological examination showed the presence of a dendritic ulcer in the right cornea. Topical therapy with an ophthalmic preparation of acyclovir was commenced and then IV acyclovir, 5 mg/kg was given 8 hourly for 5 days.

DISCUSSION

The events leading to the death of patients with pemphigus were analysed by Savin (1979). Infections such as generalized herpes simplex can contribute to the morbidity and mortality, not only from the systemic effects of a virus infection, but by aggravating the pemphigus, which then needs more intensive chemotherapy (Keane *et al.*, 1976).

Our cases illustrate some of the features of HSV infection in pemphigus. The complaint to arouse suspicion in each case was pain, typical of early herpetic lesions, and this prompted a search for the characteristic small, grouped vesicles, often seen at the edge of the pemphigus erosions, and easily missed on gross examination (Orenstein, Castadot & Wilens, 1974). Virological confirmation was obtained by the isolation of HSV from swabs and urgent reporting of provisional results is important. Even more rapid results could be achieved by submission of vesicle fluid or tissue scrapings for examination by electron microscopy within the hour. Two of our patients had had a previous herpetic infection (cold sores in case 2 and a dendritic ulcer in case 4). Even so the HSV spread widely over the skin affected by pemphigus and isolates were obtained from multiple sites in the cases (2 and 4) with extensive skin involvement. Case 3 had the most severe and prolonged generalized herpes infection. This was almost certainly a primary infection as he had a 32-fold rise in antibody titre during his illness.

Topical idoxuridine was the only acceptable anti-viral therapy for herpes infection in the skin until the advent of the relatively safe systemic preparation vidarabine, and later the more potent systemic acyclovir. Case 1 did not require systemic therapy. Case 2 was treated with vidarabine and responded well from a virological viewpoint, no further virus isolates being obtained. Vidarabine has to be infused as a 500-fold dilution of the micro-crystalline suspension in which it is supplied (25 ml/kg when dosage of 10 mg/kg is used).

Acyclovir is now the most potent anti-herpes agent and has been used in a number of clinical trials. It is relatively safe and effective in primary genital herpes (Mindel *et al.*, 1982) and in mucocutaneous herpes in the immunosuppressed (Mitchell *et al.*, 1981). We were able to obtain acyclovir for the treatment of patients 3 and 4: case 4 responded well, with no problems, and virus shedding ceased by the 4th day: case 3 was still shedding virus (sensitive to acyclovir) after 5 days of treatment at the recommended dose of 5 mg/kg 8 hourly. Pain was relieved early, however, and no new lesions appeared when the dosage was altered to 10 mg/kg, all lesions being healed by the 10th day. This patient had a rise in blood urea during treatment, a recognized hazard of bolus injections of the higher dose of acyclovir. It is now recommended that infusion is made over 1 hour.

Herpes simplex virus is an ever-present hazard to the patient with skin diseases such as pemphigus. There is a need to be alert to the signs of such infection, to use the best rapid viral diagnosis techniques for confirmation and the safest most effective antiviral therapy available.

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Correspondence

Multiple pyogenic granulomata occurring during etretinate therapy

SIR, We have observed the development of multiple pyogenic granulomata in two patients receiving etretinate therapy and we suggest this may be an unusual side-effect of this drug.

Case 1. A Caucasian male, aged 25, presented in 1979 with a 5-year history of psoriatic arthropathy and flexural psoriasis which slowly became widespread. The arthropathy progressed to such an extent that he was almost unable to walk, and bilateral hip replacement was planned when control of the psoriasis had been achieved.

This proved particularly difficult as he was intolerant of dithranol and tar preparations. Methotrexate cleared the psoriasis completely and he was maintained on a dose of 10 mg i.m. weekly but in July 1981 he developed abdominal pain, diarrhoea and melaena with a fall in haemoglobin. Endoscopy suggested Crohn's disease and the methotrexate therapy was stopped. The psoriasis virtually cleared and bilateral total hip replacements were performed. Mild side-effects included cheilitis and when the psoriasis was clear he developed a weeping intertrigo in both groins. Abduction of the thighs was somewhat limited in spite of the arthroplasty. During this period he developed several slightly painful 1-cm diameter, readily bleeding nodules on the medial aspects of both thighs (Fig. 1), scrotum and on the inferior aspect of the penis.

The lesions were biopsied and samples were sent for histology, bacteriology, mycology and culture. The histology (Fig. 2) showed the epidermis had been replaced by very vascular non-specific granulation tissue with inflammatory infiltration extending through the dermis and into the subcutaneous fat. The lesions resembled multiple pyogenic granulomata rather than granuloma gluteale. Professor E.Wilson Jones confirmed that most of the sections showed non-specific oedematous granulation tissue with the presence of eosinophils around empty tissue vacuoles. There was a dermal scar as well as surface granulation tissue,

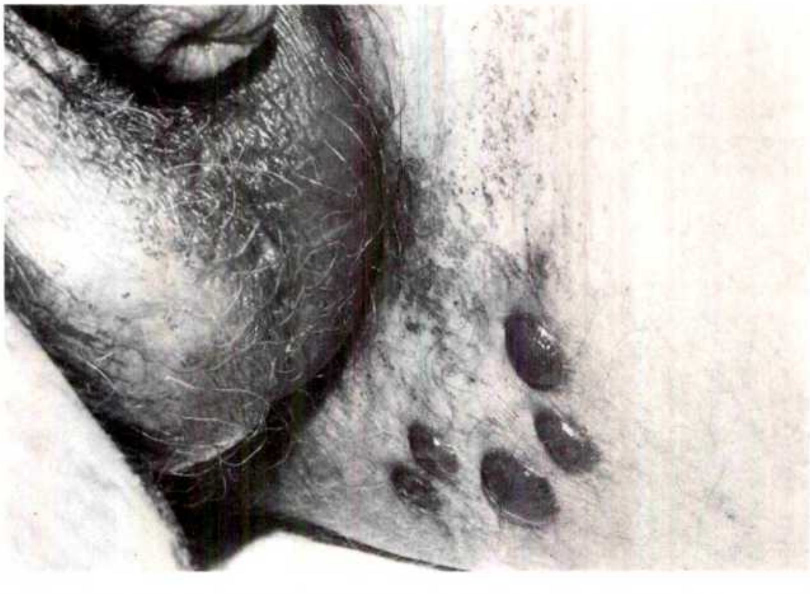

FIGURE 1. Multiple pyogenic granulomata on left upper thigh in case 1. Lesions were also present on the scrotum and penis.

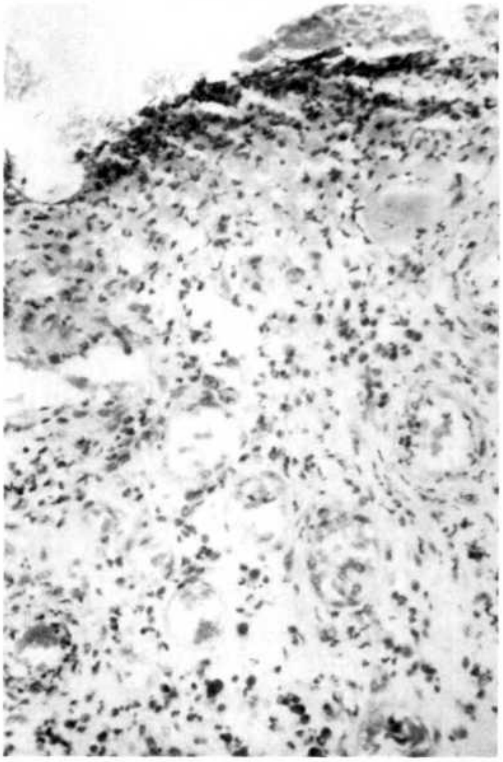

FIGURE 2. Histology of case 1, showing vascular non-specific granulation tissue with tissue lacunae (× 60).

and it was noted that a collection of cocci was present in the deepest tissue showing incipient suppuration. This type of histology raised the question of whether there might be a disorder of neutrophil function. Other sections were shown to Dr A.McQueen who commented that the lesions consisted of a focal vasculitis affecting medium-sized vessels and showing an acute process in places and in others a more granulomatous process. Surface culture from the lesions showed *Staph. albus*, *B. proteus*, *Ps. pyocyanea* and *Ent. cloacae*. Culture of the biopsy produced *B. proteus* and *Strep. faecalis*. Mycological culture was negative. HLA typing showed HLA 211 712. Other relevant investigations, including WR were negative or normal.

Case 2. This was a 72-year-old male who had intractable acrodermatitis continua, affecting the hands and feet. The condition had been so severe that he had previously had amputations of three fingers and a thumb. He was started on etretinate 25 mg b.d. and after 6 months of reasonable improvement he developed multiple lesions suggestive of pyogenic granulomata on the left forefinger and left big toe in areas that had not previously been affected by the acrodermatitis. No biopsy was taken. The lesions failed to respond to curettage or application of daily silver nitrate. There was slight improvement in the lesions when the dose of etretinate was reduced to 25 mg daily and the acrodermatitis remained under reasonable control.

In each case, we thought that the occurrence of pyogenic granulomata could be related to the etretinate therapy. Microbrasion of the affected skin might be a causative factor. The friability of the skin which occurs during etretinate therapy may have allowed micro-organisms to enter. A further factor may be that etretinate significantly inhibits the migration of neutrophils from blood to tissues when applied into skin chambers or when given orally to normal volunteers (Dubertret, Lebreton & Touraine, 1982).

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We are grateful to Professor E.Wilson-Jones, Dr A.McQueen and Dr M.Aslam for their histological opinion in case.

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REFERENCE

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Cryosurgery in cutaneous leishmaniasis

SIR, Drs Jolliffe and Bryceson (*British Journal of Dermatology* 1983, **109**, 489) have questioned the statement in our article (Bassiouny *et al.*, 1982) that 'adequate cryotreatment of cutaneous leishmaniasis will preclude the development of mucocutaneous extension' and they presumed that we are referring to South American cutaneous leishmaniasis due to *Leishmania braziliensis braziliensis.* I believe they have misinterpreted our remarks. We did not say in our paper that we have any experience with mucocutaneous leishmaniasis due to *Leishmania braziliensis braziliensis* as it has never been recorded in Saudi Arabia. A few cases of direct extension of CL to the neighbouring mucosa caused by *Leishmania tropica* have been reported. In the described cases ulceration extended from the skin on to the lips or nose (Cardamatis & Mellisides, 1911; La Cava, 1912). Trifilo (1939) cited a case of *Leishmania tropica* ulcer on the tongue. On the other hand, I must point out clearly that cryosurgery will never alone be a curative treatment in mucocutaneous leishmaniasis due to *Leishmania braziliensis braziliensis.* Systemic treatment with antimonials or amphotericin B is the fundamental therapy for that organism and cryosurgery is suggested to be used as an *adjunctive* or *palliative* measure.

I agree with Drs Jolliffe and Bryceson that 'parasite typing is likely to be useful in leishmanial therapy'. Unfortunately, techniques to differentiate the species are time-consuming and available to very few investigators. Decisions about patient management often have to be made in the absence of such information, and such investigation must not delay the patient's access to the treatment in endemic areas (Marsden, 1979).

I am concerned by the claim of Drs Jolliffe and Bryceson that in my arguments against the use of systemic antimony I misquoted one of them (Bryceson, 1970) who used the drug in prolonged high dosage for the refractory diffuse CL. They stated that the disease was the cause of scarring, not the antimonial. Nowhere did we suggest this. Of course, the scarring was due to the disease not the antimonial because in his article (p. 375) he stated that there was never any response to antimonials even at high doses approaching toxic levels (37–62 mg/kg) in the twenty-five cases treated.

Drs Jolliffe and Bryceson concluded that infections caused by *L. major*, *L. tropica* and *L. ethiopica* differ in their response to antimonials. During the last year we have extended our work and several patients with oriental sores were cured by CO_2 cryotherapy with excellent cosmetic results after they had exhausted the conventional pentavalent antimony injections for 2–3 courses (20 mg/kg). These cases were treated by applying the probe until the ice-ball reaches up to 2 mm outside the lesion margin and this generally needed 20–40 sec, markedly reducing the time of freezing already published. The 60–90 sec was not needed even for big lesions. A single freeze–thaw cycle was used and a second and third refreeze was not needed in the whole series. It is well known that the ultrastructural changes in the host cells are less marked in the one-cycle method (1 min) than the two-cycle method (1 min twice) (Ueda, Komori & Wakabayashi, 1982).

Reduction of the time of freezing and avoidance of repeated freezing are the most important two factors in minimizing cryonecrosis and trauma to host cells. The resulting negligible scar formation is possibly because cryotherapy leaves a relatively intact collagenous framework (Holden, 1975). Therefore, I still maintain that all types of leishmaniasis are thermosensitive and this has been documented in a large number of reports (Do Amaral, 1950; Pereira, De Castro & De Mello, 1958; Bell, 1968; Zeledon, 1971; Rodband 1979). The results of our new electron microscopic study of the effects of cryosurgery in CL which has been completed in the last 2 years in Saudi Arabia will soon be published. This will add further evidence for the efficacy of the one-cycle cryotherapy in 20–40 sec as a simple effective treatment. We do not think that this effect has been described before.

Although controlled clinical trials would be ideal, it was difficult in our study to include untreated control subjects as most of the patients were afraid to wait for spontaneous cure. Their fear was due to the disfiguring scars that follow delayed spontaneous healing of oriental sores in their endemic area. This sometimes lasts several years.

In our published article we chose cases which had persisted for 1 to 3 months to avoid any argument that spontaneous cure may explain the cure rate in our series. In the last year several cases have been successfully treated by cryotherapy where the duration of the lesion has varied from 1 to 3 years and in one case the duration was about 12 years. Sixty per cent of the cases had multiple lesions. Spontaneous cure if and when it occurs in CL has been observed over varying periods of time, from 6 to 7 months to 1, 14, 30 and 32 years from onset. This has been documented in a large number of reports listed in our paper.

Finally, I would like to stress that cryotherapy is a simple, rapid, effective, once-only treatment for cutaneous leishmaniasis which can be used as an alternative to antimonial therapy, especially in areas where hospitalization and courses of systemic drugs are inappropriate.

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Training in Dermatology in the United Kingdom

SIR, I was interested to read the results of the opinions of Dowling Club members concerning training in dermatology in the United Kingdom (Dowd & Lovell, *British Journal of Dermatology* 1983, **109**, 235).

It is encouraging to know that a large proportion (68%) of the registrars in Dermatology would consider that training in venereology should be obligatory. Unfortunately, on a practical basis, eight sessions of venereology could hardly be considered adequate experience; conversely, in my specialty I would not consider that eight sessions of dermatology would be adequate experience for a trainee venereologist. Venereology, which is commonly practised as part of the greater specialty of dermato-venereology within the European Economic Community, shares so much in common with dermatology. I would estimate that over one-third of conditions that a genito-urinary physician sees are sexually transmissible conditions with dermatological manifestations, many of which, of course, in the long term nowadays may have serious systemic sequelae. To take as examples: manifestations of early syphilis, the gonococcal dermatitis-arthritis syndrome, Reiter's syndrome, genital condylomata acuminata and genital herpes, as well as less serious conditions such as various causes of balanitis, vulvo-vaginitis, molluscum contagiosum and all the other myriad non-venereal diseases of the genitals. Both venereologists and dermatologists will see manifestations of the Acquired Immuno-Deficiency Syndrome, AIDS, and the management of this condition will certainly require co-operation between the two specialties.

Certainly in my department we have always had a most close and cordial co-operation with our dermatological colleagues. I may be in a minority within my specialty in the United Kingdom in stating that I wish more venereologists co-operated more intimately with their dermatological associates.

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Book Reviews

The Management of Leg Ulcers. T.J.RYAN (1983) Oxford: Oxford University Press. Pp. 91. Price £5.95.

This small book concentrates on the dermatological aspects of leg ulcer management. It claims that if the skin is healthy, the ulcer will usually heal. The aetiology of varicose ulceration and logical advice for its treatment are offered in a lively manner. It is not quite clear whether this volume is intended mainly for nurses, general practitioners or specialists. This omission is probably intentional because it is meant for the use of all who are concerned with the management of leg ulcers. However, it can be usefully read through in a couple of hours and the many helpful tips and advice on management will be of particular interest to general practitioners and district nurses.

I.SARKANY

Lecture Notes on Dermatology. BETHEL SOLOMONS (1983) (5th edition). Oxford: Blackwell Scientific Publications. Pp. 253. Price £7.50.

During the past few years there has been a welter of dermatology books placed on the market to cater for the needs of the undergraduate and the trainee in general practice. It is a tribute to its value that *Lecture Notes* has stood the text of time and has now reached its fifth edition since 1965. The text, although necessarily brief, is adequate and covers most of the common and slightly less common dermatoses; it is not pretentious. At the same time, it manages to be interesting and readable. The black and white photographs have been selected carefully and fit in well with the text. The colour pictures are of good quality and almost all have a useful point to make. The first chapter is rather pedestrian. It could be rewritten so that structure and function and their alterations are more closely linked. The definitions of histopathological terms are not useful and the section on immunology is neither one thing nor the other. There is insufficient stress placed on iatrogenic disease in general and on topical steroid abuse in particular. Indeed, there might be, with benefit, a section on recent advances in the mismanagement of skin disease. As expected, the section on leprosy is a masterpiece in miniature, but some balance is perhaps lacking when it is given as much space as psoriasis.

These minor criticisms apart, I like *Lecture Notes* and will happily recommend it to undergraduates and trainees.

M.A.COWAN

Microbial Skin Disease:Its Epidemiology. W.C.NOBLE (1983) London: Arnold. Pp. 144. Price £15.00.

The author is Professor of Microbiology at the Institute of Dermatology in London. Those familiar with his monograph *Microbiology of Human Skin* will ask how this new title differs. It is shorter and is intended for a wider audience, with the aim of providing a concise account of skin microbial ecology and epidemiology for those requiring a background knowledge of the subject. There is, not unreasonably, material common to both volumes, but the present book lays more emphasis on epidemiology and on the effects of impaired immunity. It is appropriately devoid of the taxonomic and biochemical detail so off-putting to the uninitiated. However, readers for whom some topics are too briefly discussed will appreciate the twenty-page bibliography.

The text is divided into five parts: microbiology of human skin, endogenous infection, exogenous infection, infection in the abnormal host, and external factors in infection. Although there is manifest difficulty in dividing, for example, staphylococcal infections satisfactorily between endogenous and exogenous types, the classification works fairly well and is appropriate to the theme of the book. Jargon is commendably infrequent although 'GC%' and the puzzling concept of 'virus-negative' herpes simplex went unexplained. The style is pleasantly varied and concise, typographical errors are rare and the layout of text and figures is clear.

Clinical aspects are considered briefly and generally helpfully, but the practising dermatologist might question the implied synonymity between lupus vulgaris and warty tuberculosis. The careful and well-referenced interpretation of hypotheses and published findings, applied so successfully throughout most of the book, was less impressive in the treatment of anti-staphylococcal IgE and Kaposi's varicelliform eruption.

These quibbles should not detract from overall approval of a well-executed work which successfully achieves its purpose.

A.S.HIGHET

Colour Atlas of Paediatric Dermatology. By J.VERBOV AND N.MORLEY (1982) Lancaster: MTP Press. Pp. 157. Price £32.50.

The two authors of this atlas of paediatric dermatology are both very experienced dermatologists with a special interest in paediatric disorders. It is not surprising to find, therefore, a wide range of conditions represented in this atlas. Some conditions are very well represented, for example, there are five pictures of a collodion baby.

The success of an atlas such as this will depend on the quality of the photographs, and generally speaking, they are good. However, they are quite variable, and some pictures appear to be somewhat over-exposed. Most of the pictures have been carefully selected to illustrate a particular point of the condition. They are also comprehensive, with most of the important paediatric conditions well represented.

Paediatric dermatology must be regarded as a Cinderella area of dermatology in this country, and only comparatively recently have there been a number of experienced dermatologists taking a special interest in the subject, although in the United States it is a well recognized area of dermatological practice. For people such as these, this atlas will fill a gap and provide them with good pictures. It will also be a useful addition for many libraries with books on dermatology. However, the text is minimal and the book could not be bought for the text alone. For general practitioners and medical students the price would be prohibitive and many of the conditions are too rare to justify purchase. Paediatricians may find it a useful reference book, if they wish to identify a particular condition.

R.S.WELLS

British Journal of Dermatology (1983) **109**, 622.

News and Notices

Cutaneous Melanoma Course, Boerhaave, April 1984

This course, to be held at Boerhaave, The Netherlands on 12–13 April 1984 will review fundamental aspects, diagnosis, prognostic factors and therapy of cutaneous melanoma. The course is intended for surgeons, oncologists, dermatologists, pathologists and research workers in the melanoma field. Further details may be obtained from: Boerhaave Commissie for Postgraduate Teaching, Academisch Ziekenhuis, Rinjsburgerweg 10, 2333 AA, Leiden, The Netherlands.

Malignant Melanoma Symposium, Palm Springs, California, April 1984

A symposium on malignant melanoma will be held at the Riviera Hotel, Palm Springs, California in April 1984. The speaker will be A.Bernard Ackerman, M.D. For more information contact Charles Steffen, M.D., Palm Springs Medical Center, 1695 North Sunrise Way, Palm Springs, CA 92262, U.S.A.

International Symposium, Norway, June 1984

The Second International Symposium will be held at the Hotel Alexandra, Loen (situated at the Nordfjord), Norway on 17–20 June 1984. The scientific programme will include different aspects of atopic dermatitis. For information contact Professor Georg Rajka, Dept. of Dermatology, Rikshospitalet, Pilestredet 32, Oslo 1, Norway.

Stiefel Travelling Grants

We are pleased to announce that through the generosity of Stiefel Laboratories (U.K.) Ltd. Travelling Grants for 1983 have been awarded to Dr P.S.Friedman, Dr J.J.Horton, Dr C.B.Archer and Dr J.N.Leonard. Applications are once more requested, and application forms and regulations can be obtained from the Honorary Secretary, British Association of Dermatologists, 7 John Street, London, WC1N 2ES.

B.A.D. Travelling Fellowships

Travelling Fellowships were awarded to Dr J.Miller, Dr R.H.Meyrick Thomas, Dr Rachel Greenwood and Dr J.Harper for 1983. Applications are invited for these Fellowships which will be awarded for a period of not less than 3 weeks. It is anticipated that Fellows would visit various centres either in the U.S.A. and Canada, or in Europe, and attend one of the national meetings in dermatology. A grant will be given towards expenses. Application forms may be obtained from the Honorary Secretary, British Association of Dermatologists, 7 John Street, London, WC1N 2ES. Closing date: 31 January 1984.

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Stafford Miller Ltd. Professional Relations Division, Hatfield, Hertfordshire AL10 0NZ

Reference: 1. Medicine 1977; 32: 185

Essentials of Skin Disease Management

D.J. Barker MA, MB, MRCP, *Lecturer in Dermatology, University of Leeds and Honorary Registar in Dermatology, The General Infirmary at Leeds*

and **L.G. Millard** MB, MRCP, *Consultant Dermatologist, Rotherham Area Health Authority and Clinical Lecturer, University of Sheffield*

From the reviews of this practical handbook

'For general practitioners, dermatologists and medical students the book is a must.'
British Medical Journal

'A thoroughly sensible and useful pocket-sized book on the management of skin disease.'
Clinical and Experimental Dermatology

'I would recommend this book to GPs, housemen, tutors and schools of nursing as a useful addition to the library.' *Nursing Times*

'A valuable little book which is lucid, surprisingly comprehensive and rounded off with a sound basic formulary.' *British Journal of Hospital Medicine*

1979. 96 pages, 25 illustrations. Paper, £5.50

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Lecture Notes on Dermatology

New Edition

Bethel Solomons MA, MD, FRCPI
Consultant Dermatologist to Chelmsford and Essex General Hospital, Herts and Essex General Hospital and Hertford County Hospital

From the reviews of previous editions

'This is a well written and presented little book and can be recommended to all those in the profession who wish to have a working knowledge of the subject.' *On Call*

'"Lecture Notes" is a useful pocket sized dermatology text book which lists and describes the most common conditions including leprosy and syphilis and has a large collection of both black and white and colour photographs which are of good quality.'
British Journal of Dermatology

The fifth edition has again been extensively revised and updated and will continue to be invaluable to students and general practitioners.

Fifth Edition, 1983. 262 pages, 108 illustrations. Paper, £7.50

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Dermatological Nursing and Therapy

R.H. Seville MD, FRCP, *Consultant Dermatologist,* and
E. Martin, *Senior State Enrolled Nurse, both of the Beaumont Hospital, Lancaster*

'The general nurse . . . will find this book a helpful guide . . . designed for use on the Joint Board of Clinical Nursing Studies course in dermatology . . . well illustrated, with text uncluttered by any jargon. There is an excellent dermatological formulary at the end of the book.' *Nursing Mirror*

Contents: Dermatological nursing; Psychosomatic dermatology; Psoriasis, introduction to dressings; Eczema, infantile eczema; Prurigo, neurotic, excoriations, artefacts, trichotillomania, alopecia areata, lichen planus; Seborrhoeic dermatitis, acne, rosacea, ano-genital pruritis; Urticaria and drug eruptions; Dermatitis; Immunology, blistering diseases; Cutaneous manifestations of systemic disease and malignancy; Granulomata including syphilis; Infections — bacterial, fungal and viral; Infestations; Leg ulcers; Some problems in the elderly; Tumours — benign and malignant; Procedures — biopsy, therapeutic and cosmetic; Investigations — histological, mycological and bacteriological; PUVA, X- and Grenz-ray therapy, physiotherapy, chiropody, dietetics; The dermatology team; Structure, function and care of the skin, nails and hair; Principles of physical treatment and preparations to avoid.

1981. 236 pages, 113 illustrations. Paper, £7.50

Blackwell Scientific Publications

Essentials of Industrial Dermatology

Edited by **W.A.D. Griffiths** MD, MRCP
Consultant Dermatologist, St. John's Hospital for Diseases of the Skin and Senior Lecturer, Institute of Dermatology, London

and **D.S. Wilkinson** FRCP
Consultant Dermatologist, Wycombe General Hospital

This compact handbook provides guidelines and sets out basic principles in the field of industrial dermatology for doctors working as full- or part-time factory doctors, who may have little previous dermatological training. It will also appeal to doctors working in public or community health, particularly in the developing countries where dermatological problems are becoming increasingly apparent. The text includes line drawings and tables for easy reference.

Contents: A national problem *W.A.D. Griffiths;* Industrial dermatitis today and its prevention *D. Burrows;* Constitutional skin diseases in industry *J. Cotterill;* Investigation of industrial dermatitis *C. Hindson;* Soluble oils *R. Rycroft;* Hand eczema in industry and the home *R. Church;* Chemical acne *D. Williamson;* Principles in dermatitis litigation *R. Bursell;* Conclusions and key points *D.S. Wilkinson & W.A.D. Griffiths.*

Spring 1984. 150 pages, 20 illustrations. About £10.00

Blackwell Scientific Publications

International Journal of Cosmetic Science

Edited by J.M. Blakeway, Roure Bertrand Dupont S.A., 55 Voie des Bans, Boite Postale 24, 95102 Argenteuil Cedex, France

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NOTICE TO CONTRIBUTORS

The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes signed editorials reviewing, primarily for the benefit of the clinician, advances in laboratory research, and also reviews of recent advances in such aspects of dermatology as contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews, correspondence and society proceedings. The Journal is the official organ of the British Association of Dermatologists and also of the Netherlands Association for Dermatology and Venereology, but it attracts contributions from all countries in which sound research is carried out, and its circulation is equally international.

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Manuscripts should be sent to the Editor, Dr J.L.Burton, Dermatology Department, Bristol Royal Infirmary, Bristol BS2 8HW. They should be typewritten on one side of the paper only, with a wide margin, be double spaced, and bear the title of the paper, name and address of each author, together with the name of the hospital laboratory or institution where the work has been carried out. The name and full postal address of the author who will be responsible for reading the proofs should be given on the first page. A duplicate copy, complete with figures, tables and references, should be sent, since in many cases two referees are consulted. The author should keep a carbon copy of his manuscript. The Editorial Board reserves the right to make literary corrections.

Abbreviations and units. Full names with uncommon abbreviations must be given with the first mention: new abbreviations should be coined only for unwieldy names and should not be used at all unless the names occur frequently. In the title unusual abbreviations should be identified, in the introduction and discussion they should be used sparingly. Abbreviations of units should conform with those shown below.

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milligram(s) (10^{-3}g)	mg	second(s)	s	gravitational acceleration	***g***
microgram(s) (10^{-6}g)	μg	cubic millimetre(s)	mm^3	micrometre(s)	μm
nanogram(s) (10^{-9}g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12}g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^{131}I
hours(s)	h	milliequivalent	mmol		

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Page proofs will be submitted without the original typescript to the author for proof-correction (see under 'manuscripts') and should be returned to the Editor within three days. Major alterations from the text cannot be accepted.

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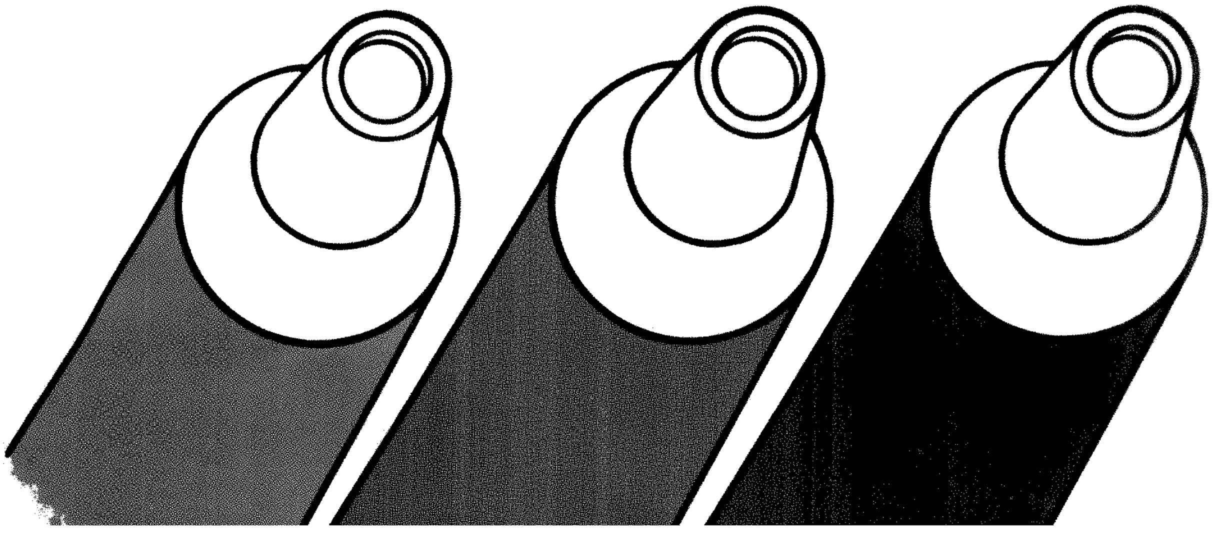

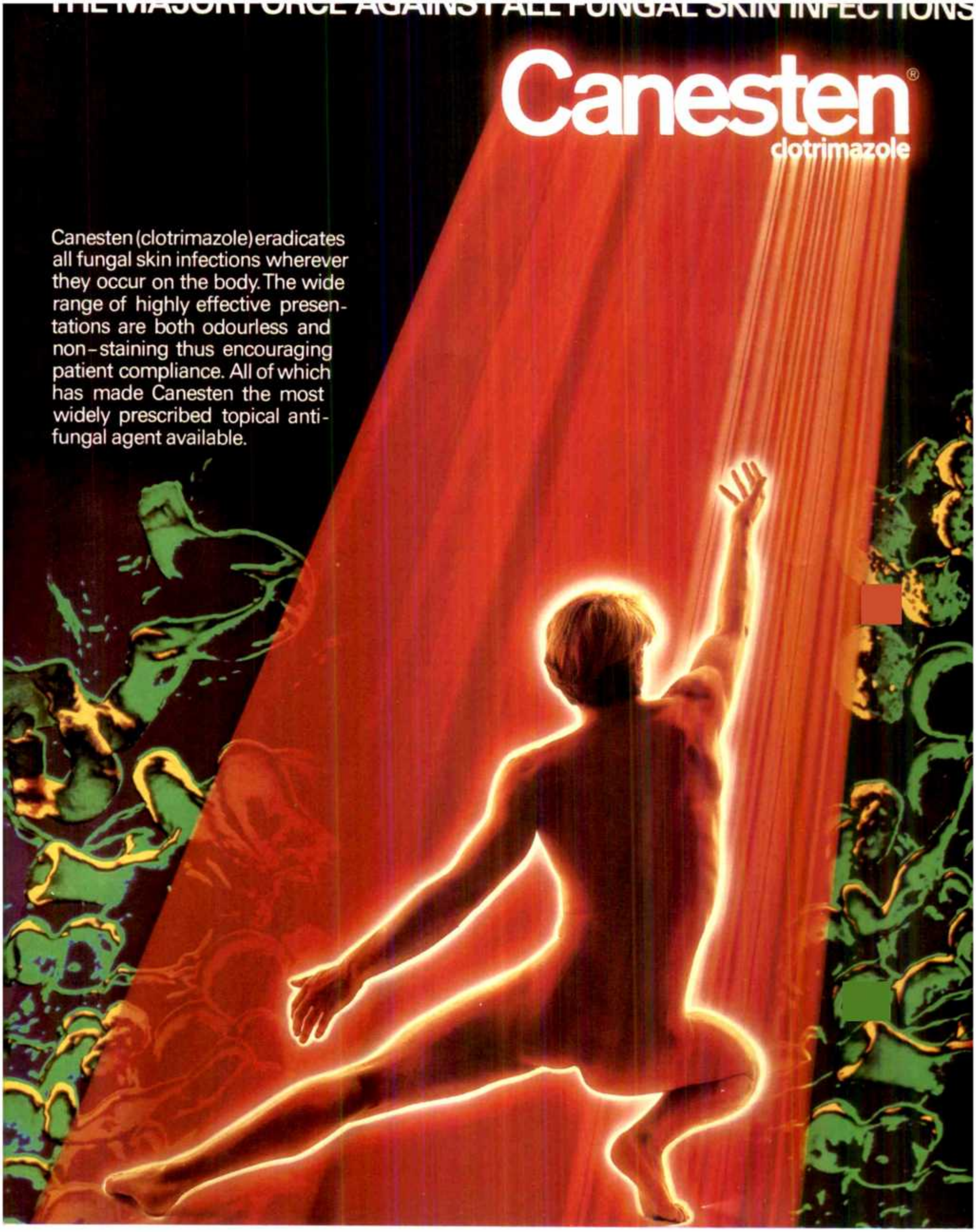
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d Administration *Cream, Atomiser Spray, Solution* Apply to the affected area 2 – 3 times daily. *Powder* as above and may also be dusted inside articles
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perience a transient local mild burning or irritation. **Precautions** Canesten atomiser spray should not be used near naked flame, should not be allowed
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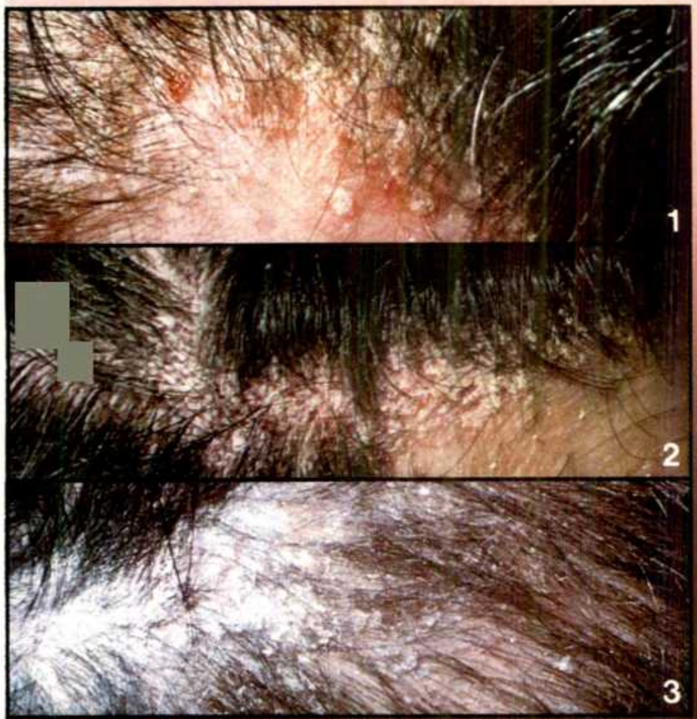

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Uses Polytar Liquid and Polytar Plus are indicated in the treatment of scalp disorders such as psoriasis, seborrhoea, dandruff, eczema and pruritus. They are also of value in the removal of pastes and pomades used in the treatment of psoriasis.

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Contra-indications, warnings, etc. Nil

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Product Licence Number
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[1] **Mims Magazine, 15th May 1982**

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Fry L. Br Med J 1982; 284:1093–109

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de effects

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ESTABLISHED: July, 1979 **PUBLISHED:** Monthly **EDITOR:** J. Graham Smith, Jr., M.D.

The **JOURNAL OF THE AMERICAN ACADEMY OF DERMATOLOGY** is a professional journal devoted exclusively to the clinical and continuing education needs of the entire dermatologic community, including those family practitioners, pediatricians, and internists who are concerned with clinical manifestations of skin disease in their practice.

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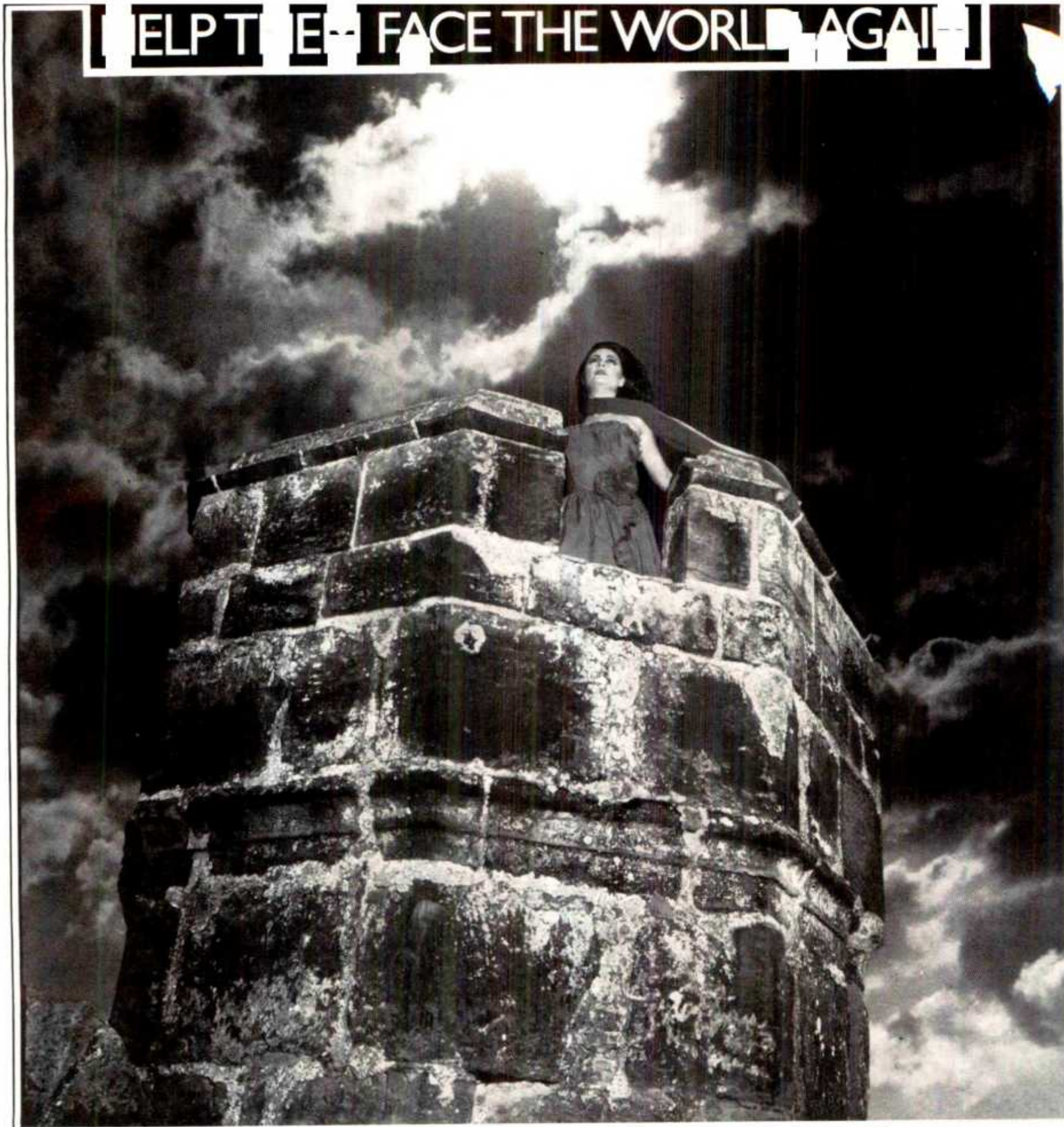

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Reference: 1. Medicine 1977; 32: 18

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1979. 96 pages, 25 illustrations. Paper, £5.50

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Edited by **W.A.D. Griffiths** MD, MRCP
Consultant Dermatologist, St. John's Hospital for Diseases of the Skin and Senior Lecturer, Institute of Dermatology, London

and **D.S. Wilkinson** FRCP
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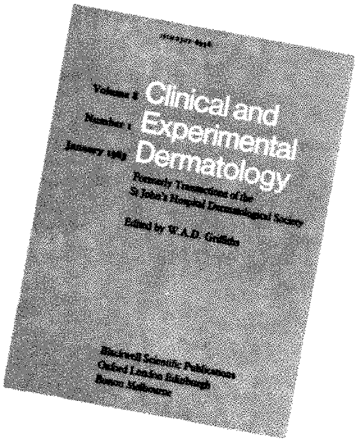

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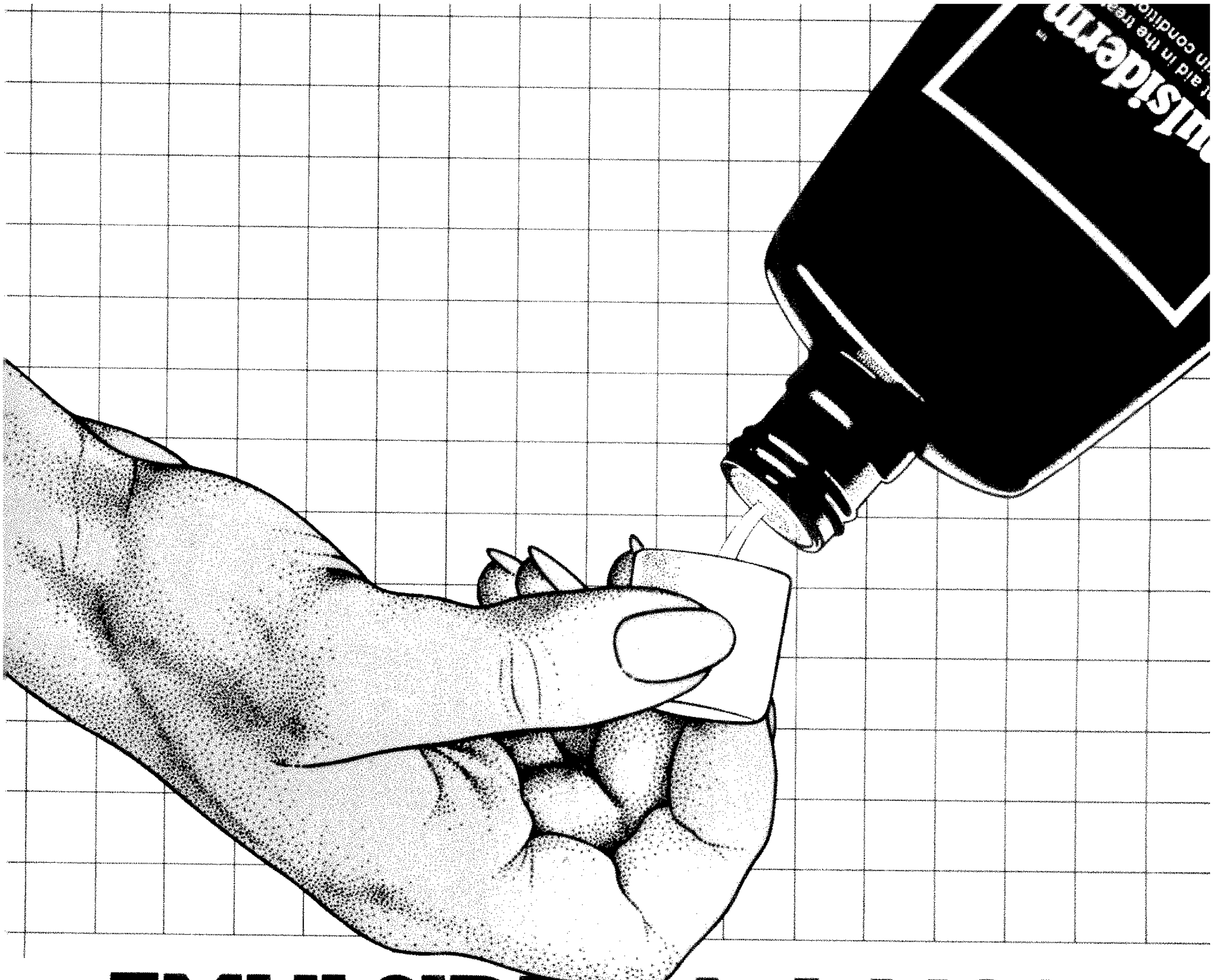

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British Journal of Dermatology (1983) **109,** 623–633.

Clinical and Laboratory Investigations

Distribution of major histocompatibility antigens in normal skin

T.J.HARRIST, D.J.RUITER*, M.C.MIHM, JR AND A.K.BHAN

Immunopathology and Dermatopathology Units, Department of Pathology, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts, U.S.A. and *Department of Pathology, University Medical Centre, Leiden, The Netherlands

Accepted for publication 5 May 1983

SUMMARY

The distribution of major histocompatibility antigens HLA-A,B,C (HLA), β_2-microglobulin (β_2m), and Ia-like antigens (HLA-DR; Ia) in normal skin was studied in frozen tissue sections by a four-step immunoperoxidase method and an avidin–biotin method employing monoclonal antibodies. HLA and β_2m were present on the basal and spinous keratinocytes of the epidermis, on the outer root sheath epithelium in the infundibulum of the hair follicle, and on the excretory sebaceous duct epithelium. Ia-positive dendritic cells were found in the epidermis and hair follicles, but they were more frequent in the infundibulum and isthmus of the hair follicle than in its inferior portion or in the epidermis. In the straight eccrine duct, HLA and β_2m-positivity was most striking in its lower portion. In the superficial duct, there was a less intense staining using the four-step procedure, but when an avidin–biotin method was used, the difference was less apparent. In contrast, the acrosyringial epithelium was markedly Ia-positive with decreasing intensity of staining as the duct penetrated the dermis. No HLA or Ia antigens were identified in eccrine glands and apocrine glands. Eccrine glands were slightly β_2m-positive. HLA and β_2m were uniformly present in non-dilated and dilated intradermal apocrine ducts.

The presence of major histocompatibility complex antigens HLA-A,B,C (HLA) and β_2-microglobulin (β_2m) on keratinocytes and of Ia-like (HLA-DR, Ia) antigens in dendritic cells of the epidermis is well known (Ackerman-Schopf, Ackerman & Terasaki, 1974; Governa & Biguzzi, 1976; Saurat, Gluckman & Didierjean, 1977; Rowden, Lewis & Sullivan, 1977; Korthals Altes-Levy van Vinnighe & Neuman, 1981; Turbitt & MacKie, 1981; Murphy *et al.*, 1981; Natali *et al.*, 1981). However, these studies dispute the exact localization of HLA and β_2m in the epidermis. For example, β_2m, the light chain of HLA, was found only in the basal layer in one study (Governa & Biguzzi, 1976) and throughout the entire epidermis in others (Korthals Altes-Levy van Vinnighe & Neumann, 1981; Turbitt & MacKie, 1981). The exact distribution

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of these antigens in normal skin is relevant because changes in expression of HLA and β_2m in premalignant and malignant skin lesions have been described (Korthals Altes-Levy Van Vinnighe & Neuman, 1981; Turbitt & MacKie, 1981; Ruiter *et al.*, 1982). Furthermore, the distribution of HLA and β_2m in epidermal adnexae has not been well documented.

Monoclonal antibodies against major histocompatibility antigens have now been developed (Bhan *et al.*, 1980; Ruiter *et al.*, 1982), and a sensitive four-step immunoperoxidase technique (Bhan *et al.*, 1980) and an avidin–biotin technique (Hsu, Raine & Fanger, 1981) have allowed the localization of various antigens within the thymus (Bhan *et al.*, 1980), lymph nodes (Poppema *et al.*, 1981; Bhan *et al.*, 1981), and skin (Bhan *et al.*, 1981a; Ruiter *et al.*, 1982). Therefore, we have studied the distribution of major histocompatibility antigens in normal skin with two immunohistochemical techniques and monoclonal antibodies.

METHODS

Ten excisional biopsies and/or surgical excision specimens of naevomelanocytic hyperplasia or naevocellular naevi were obtained from ten patients (five males and five females), aged from 34 to 66 years. The excision sites included the scalp, axilla, arm, abdomen, flank, back, groin, thigh, knee and toe. A representative slice through each specimen was divided into two parts, each containing a portion of the melanocytic lesion and the adjacent skin 1 cm away from the lesion. Skin from both axilla and perineum was taken from two consecutive cadavers (27-year-old male, 91-year-old female) at autopsy, 10 and 24 h after death, respectively. In addition, six 3-mm punch biopsies of clinically normal skin were taken from six healthy volunteers aged 26–35 years (four males and two females). The biopsy sites were the posterior upper arm and buttock. Each tissue specimen was snap-frozen in OCT compound (Ames Co, Elkhart, Ind.) and stored at −70°C.

Monoclonal antibodies

Monoclonal antibodies reactive with major histocompatibility antigens HLA-A,B,C (HLA) (Bethesda Research Lab., Inc., Gaithersburg, Md.), β_2-microglobulin (β_2m), and Ia-like antigens (Ia) were used (anti-β_2m and anti-Ia antibodies were kindly provided by Dr L.Nadler, Sidney Farber Cancer Institute, Boston, Mass.). Anti-HLA antibody is reactive with the common framework antigen of HLA-A,B,C. The monoclonal anti-HLA-DR antibodies employed (anti-I1 and anti-I2) recognize a non-polymorphic region of human Ia-like (HLA-DR) antigens (Reinherz *et al.*, 1979; Nadler *et al.*, 1981).

Immunoperoxidase studies

Four-micron thick frozen sections were air-dried, fixed in acetone for 10 min and stained by a four-step peroxidase–anti-peroxidase (PAP) method (Bhan *et al.*, 1981; Poppema *et al.*, 1981). Sections were first incubated with a 1 : 500 dilution of mouse ascites containing one monoclonal antibody for 60 min at room temperature followed by sequential 30-min incubations with rabbit anti-mouse immunoglobulin (Ig), swine anti-rabbit Ig, and lastly with peroxidase–rabbit Ig–anti-peroxidase reagent (Dako Immunoglobulins, Copenhagen, Denmark). After each incubation, sections were washed in three changes of phosphate-buffered saline (PBS), pH 7·4. Staining was achieved by incubation of sections in an acetate buffer solution (pH 5·0) that contained 3-amino-9-ethylcarbazole (Aldrich Chemical Co., Inc., Milwaukee, Wis.), dimethylformamide and hydrogen peroxide. The sections were washed in acetate buffer and post-fixed in acetate-buffered 4% formaldehyde for 10 min. They were counterstained with Gill's triple strength haematoxylin (Lerner Lab., New Haven, Conn.) for 10 seconds, rinsed in tap water, 'blued' in saturated lithium carbonate solution, washed in distilled water and mounted in Elvanol® (E.L. Dupont de Nemours & Co., Wilmington, Del.).

Sections were also stained by a three-step avidin–biotin technique (Vector Laboratories, Burlingame, Ca.) (Hsu *et al.*, 1981). The sections were first incubated with a 1:100 to 1:500 dilution of mouse ascites containing the monoclonal antibody for 60 min at room temperature followed by two 30-min incubations with biotin-labelled horse anti-mouse Ig and avidin–biotin–peroxidase complex respectively. Staining was achieved by incubation in an acetate buffer solution (pH 5·0) that contained 3-amino-9-ethylcarbazole, dimethylformamide and hydrogen peroxide. The sections were washed in acetate buffer, mounted in Elvanol®, and counterstained with Gill's triple strength haematoxylin.

Control sections were processed exactly as described except in the initial incubation where PBS or control mouse ascites fluid were used.

RESULTS

Sections stained with monoclonal antibodies had distinct peripheral staining consistent with cell membrane staining, cytoplasmic staining, or both. In contrast, sections incubated with control ascites fluid or PBS showed no staining, except in mast cells, which have endogenous peroxidase activity.

Epidermis (Table 1)

Marked HLA- and β_2m-positivity were present in the basal and spinous layers. In the spinous

TABLE 1. Distribution of major histocompatibility antigens in epidermis and dermis

	HLA/β_2m	Ia
Epidermis:		
Stratum corneum	−	−
Stratum granulosum	−	−
Stratum spinosum	+	−
Stratum basale	+	−
Melanocytes	NI*	−
Other dendritic cells	NI	+
Dermis:		
Stromal interstitial cells	Few +	−
Perivascular fibrohistiocytic and dendritic cells	+	+
Arteries - endothelium	+	+/−
- smooth muscle	−	−
Arterioles - endothelium	+	+/−
- smooth muscle	−	−
Capillaries - venules - endothelium	+	+
- pericytes	+	+
Nerves - Schwann cells	mostly +/some −	−
Arrectores pilorum	−†	−

* NI = Not interpretable.

† Interstitial staining, not that of smooth muscle cells.

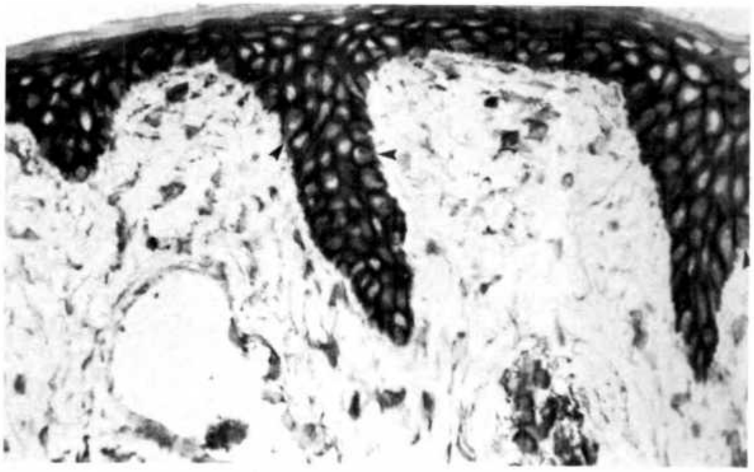
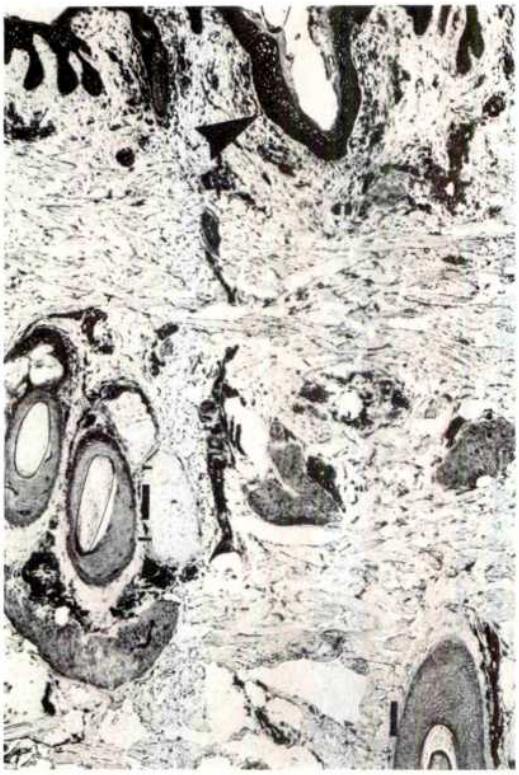


FIGURE 1. Normal skin stained with anti-HLA antibody. Both basal and spinous layers of the epidermis have a peripheral staining pattern. Note the absence of HLA staining on the basement membrane zone side of the basal cells (arrowheads). The stratum granulosum and stratum corneum have no immunostaining (× 340, Gill's triple strength haematoxylin).

TABLE 2. Distribution of major histocompatibility antigens in the pilo-sebaceous units

	Staining for		
	HLA	β_2m	Ia
Terminal hair follicle:			
Papilla	–	–	–
Matrix	slight +	slight +	–
Inner root sheath	–	–	–
Outer root sheath, deep portion:			
Epithelium	–	–	–
Dendritic cells	rare +	rare +	few +
Outer root sheath, superficial portion and isthmus:			
Epithelium	+	+	–
Dendritic cells	NI*	NI*	many +
Sebaceous apparatus:			
Gland:			
Generative epithelium	–	–	–
Sebaceous cells	–	–	–
Dendritic cells	rare +	rare +	rare +
Excretory duct:			
Epithelium	+	+	–
Stratum corneum	–	–	–
Dendritic cells	NI*	NI*	Occasional +

* NI = Not interpretable.



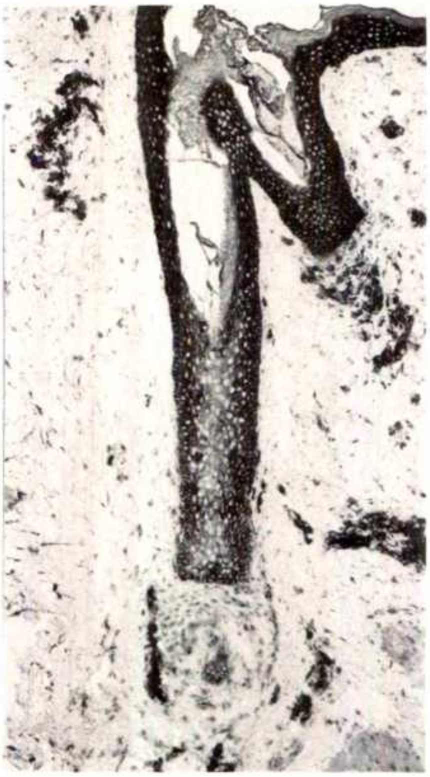


FIGURE 2. (Left) Normal skin stained with anti-HLA antibody. The outer root sheath epithelium stains markedly in the infundibulum (arrowhead), but is minimal to absent in the inferior portion (I) (× 60, Gill's triple strength haematoxylin).

FIGURE 3. (Right) Normal skin stained with anti-HLA antibody. HLA positivity extends to the papilla. (× 100, Gill's triple strength haematoxylin).

layer, the intensity diminished superficially to the level of the granular layer. The staining was predominantly peripheral, particularly accentuating plasmodesms (Fig. 1). Suprabasal dendritic cells were intensely Ia-positive in a cytoplasmic staining pattern.

Hair follicles and sebaceous apparatus (Table 2)

The epithelium of the outer root sheath had intense peripheral staining for HLA and β_2m in the infundibulum of large terminal hairs. Staining intensity markedly diminished in the superficial isthmus and was virtually absent in the inferior follicle (Fig. 2). Small catagen and telogen follicles had staining for HLA and β_2m of the complete outer root sheath epithelium (Fig. 3). The hair matrix was slightly positive for HLA and β_2m but negative for Ia. In the hair papilla, few dendritic cells exhibited cytoplasmic staining for both HLA, β_2m and Ia.

The absent epithelial staining in the inferior terminal follicles permitted recognition of rare HLA- and β_2m-positive dendritic cells in a cytoplasmic pattern. Ia-positive dendritic cells were few in the lower follicle, but there were many in the upper portion (Fig. 4).

Excretory duct epithelium had peripheral staining for HLA and β_2m. The sebaceous gland generative epithelium was negative for β_2m and HLA. In the basal layer of the sebaceous glands,

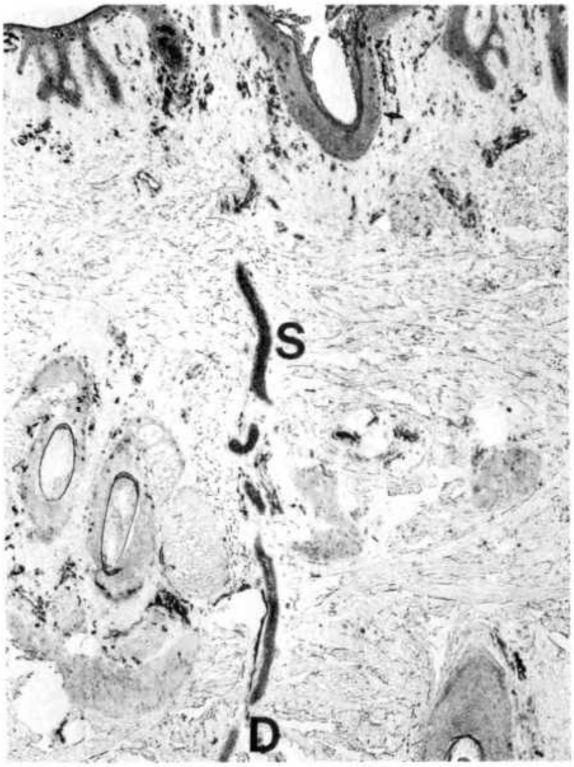


FIGURE 4. Normal skin stained with anti-Ia antibody. Many Ia-positive dendritic cells are identified in the follicular infundibulum and isthmus, while fewer are in the inferior portion. Note the marked Ia-positivity of the superficial dermal eccrine duct (S), diminishing in its deeper portion (D) (× 60, Gill's triple strength haematoxylin).

TABLE 3. Distribution of major histocompatibility antigens in sweat glands

	Staining for		
	HLA	β_2m	Ia
Eccrine apparatus:			
Gland	–	slight +	–*
Intradermal duct:			
superficial	– or trace	– or trace	+
deep	+	+	– or trace
Intraepidermal duct	–	–	+
Apocrine apparatus:			
Gland	–	–	–*
Intradermal duct	+	+	+
Dilated paraglandular duct	+	+	–*

* Rare dendritic cells were Ia-positive.

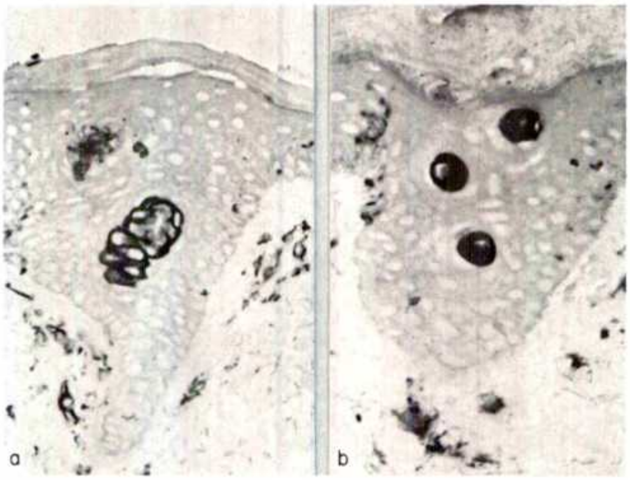


FIGURE 5. Acrosyringium stained with anti-Ia antibody (× 256, no counter stain). (a) The innermost cells have peripheral staining pattern; (b) the cuticle also is intensely stained.

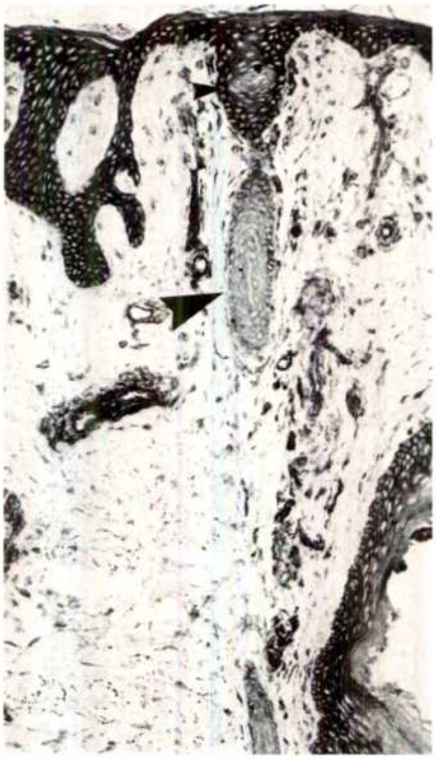


FIGURE 6. Normal skin stained with anti-HLA antibody. The acrosyringium and intradermal eccrine duct do not stain with anti-HLA (× 144, Gill's triple strength haematoxylin).

a few Ia-positive dendritic cells were observed. More Ia-positive dendritic cells were encountered in the sebaceous excretory duct than in the gland itself.

Eccrine and apocrine apparatus (Table 3)

The luminal epithelium of the superficial portion of the intradermal duct and the acrosyringium stained weakly for HLA and β_2m, but did stain intensely with Ia as did the cuticle (Figs 5 and 6). In contrast, the eccrine glands were negative for HLA and Ia, with only slight positivity for β_2m. The epithelium of the lower intradermal eccrine duct stained peripherally for HLA and β_2m, but weakly for Ia (Fig. 7).

Apocrine glands were negative for HLA and β_2m (Fig. 8), as well as negative for Ia (Table 3).

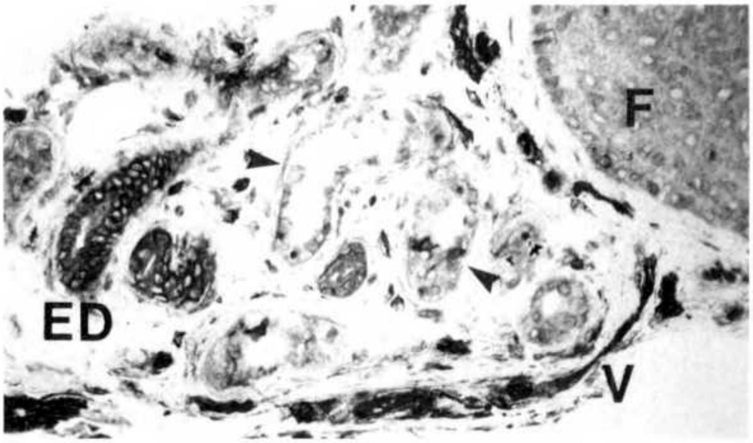


FIGURE 7. Normal skin stained with anti-HLA antibody. The lower portion of the eccrine duct (ED) is HLA-positive, in contrast to adjacent eccrine glands (arrowheads) and inferior portion of the hair follicle (F) (V = blood vessel) (× 256, Gill's triple strength haematoxylin).

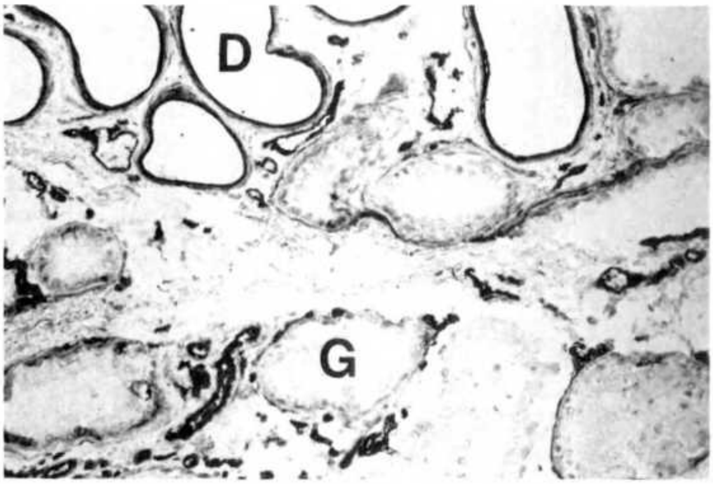


FIGURE 8. Normal axillary skin stained with anti-HLA antibody. The non-dilated and dilated apocrine ducts (D) are markedly HLA-positive, in contrast to apocrine glands (G) which are unstained (× 160, Gill's triple strength haematoxylin).

The intradermal duct stained peripherally for HLA, β_2m and Ia. Dilated apocrine ducts present in the lower part of the dermis stained for HLA and β_2m, but variably for Ia (dendritic cells).

Dermis (Table 1)

Perivascular fibrohistiocytic cells and dendritic cells stained intensely for HLA, β_2m and Ia in a cytoplasmic pattern. Fibrohistiocytic cells are elongated cells (probably fibroblasts or tissue macrophages) which are seen about vessels. Some may represent Langerhans/indeterminate cells. Endothelial cells of capillaries, apparent lymphatic vessels and venules stained intensely for HLA, β_2m, and Ia in peripheral array. Endothelium of arterioles was variably Ia-positive but was intensely stained with HLA and β_2m. Arterial endothelium was positive for HLA and β_2m and focally positive for Ia. Most Schwann cells were peripherally stained for HLA and β_2m, whereas others were not. All Schwann cells were Ia-negative. Smooth muscle in vessel walls had weak to absent staining for HLA and β_2m with no staining for Ia. In the arrectores pilorum, slight interstitial staining of HLA and β_2m was observed (Table 1).

DISCUSSION

Immunohistochemical demonstration of the major histocompatibility antigens in skin was possible even in autopsy tissue some 10–24 h after death. The basal and spinous layers of the epidermis, the outer root sheath epithelium of the upper part of the terminal hair follicle, and the excretory sebaceous duct epithelium express both HLA and β_2m. In contrast, the inferior portion of terminal hair follicle epithelium was HLA- and β_2m-negative. In small catagenic and telogenic hair, the complete outer root sheath epithelium stained for HLA and β_2m. Moreover, both eccrine and sebaceous glands also were HLA negative in contradistinction to the ducts. The presence of histocompatibility antigens in the epidermis and upper portions of epidermal adnexae may be a reflection of the role of these antigens in the expression of individual identity with respect to the external environment. This view is in concordance with the absence of staining for HLA in liver parenchymal cells and brain parenchymal cells (Fleming *et al.*, 1981).

It is conceivable that the differences in HLA expression are not qualitative but quantitative, whereby the presence of a low concentration of HLA is beneath the detection limit of the immunoperoxidase technique (Fleming *et al.*, 1981). The discrepancy in staining distributions of HLA and β_2m with that found by other workers (Ackerman-Schopf *et al.*, 1974; Governa & Biguzzi, 1976; Saurat *et al.*, 1977; Korthals Altes-Levy Van Vinnighe & Neumann, 1981; Turbitt & MacKie, 1981) may be due to differences in technique or antibodies applied. We feel that the four-step immunoperoxidase technique with counterstaining on frozen sections and the avidin–biotin technique used in the present study are more sensitive than conventional immunofluorescence. The latter technique may be more sensitive, in that β_2m-positivity was more easily demonstrable when the avidin–biotin procedure was used.

In acute graft-versus-host disease, the eccrine glands were reported to be free from lymphocytic infiltration (Slavin & Woodruff, 1974). The absence of staining for histocompatibility antigens in eccrine glands (only slight β_2m positivity) observed in our study may explain the pattern of lymphocytic invasion in graft-versus-host disease.

HLA and β_2m expression was most prominent in the lower portion of the eccrine duct with decreasing intensity of staining superficially. In contrast, the acrosyringium was markedly Ia-positive with decreasing intensity of staining down the intradermal duct. The relative absence of HLA and β_2m and the presence of Ia-antigens in the acrosyringium represents a unique situation, because tissue distribution of Ia antigens is much more restricted than HLA and β_2m. However, Natali *et al.* (1981) demonstrated expression of Ia antigens in several normal human tissues immunohistochemically with a monoclonal antibody on frozen sections. In their study, Ia expression was not restricted to cells with immune functions.

The acrosyringial epithelial cell represents another Ia-positive intraepidermal cell like Langerhans and indeterminate cells. Therefore, it is imperative that clusters of Ia-positive cells in the epidermis be carefully scrutinized before interpreting them as collections of Langerhans/indeterminate cells. Ia-positive dendritic cells were more numerous in the infundibulum and isthmus of the hair follicle than in its inferior portions or in the epidermis. The same pattern of staining was found with anti-T6 antibody, but the number of T6-positive dendritic cells always exceeded that of Ia-positive cells (Bhan *et al.*, 1981a; Harrist *et al.*, 1983; Ruiter *et al.*, 1982), indicating that T6 is a better marker than Ia for Langerhans cells and indeterminate cells using the present technique and antibodies. The absence of epithelial staining for HLA and β_2m in the sub-infundibulum permitted recognition of rare HLA and β_2m-positive dendritic cells. Application of immunoelectron microscopy has confirmed that these HLA- and β_2m-positive dendritic cells are Langerhans cells and indeterminate cells (Bronstein *et al.*, 1982). Remarkably, the number of Ia-positive dendritic cells in this portion of the hair follicle was greater than that of HLA and β_2m-positive cells.

In conclusion, a detailed description of the distribution of major histocompatibility antigens in normal skin using monoclonal antibodies on frozen tissue sections is given. The findings may be relevant for the diagnosis and histogenesis of skin adnexal tumours and for transplantation biology. We have demonstrated that Langerhans/indeterminate cells are not the only intraepidermal cells that express Ia antigens, in that acrosyringial epithelium does as well. Langerhans/indeterminate cells appear in greater numbers within follicles.

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Is upward basal cell movement independent of mitosis in the normal epidermis?

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SUMMARY

An analysis has been made of the possible mechanisms by which cells leave the basal layer distally during cell replacement in the steady state mammalian epidermis. Etoh's concept (1975) of cells actively migrating from the basal layer is challenged. Computer experiments suggest that if basal cell migration is an active process, the rate of cell migration consistent with the kinetics of the process being examined would yield a depopulation value far higher than the one observed experimentally. We found by contrast that rates of migration comparable to those found experimentally after mitosis inhibition by irradiation were obtained in our computer simulation experiments if it was assumed that distal basal cell migration is a passive event resulting from cells being extruded from the basal layer. We believe that there is not at present sufficient data to support the concept of active cell migration from the basal layer. The evidence favours the concept that in normal steady state epidermal populations, cells are passively removed from the basal layer as a result of forces generated, directly or indirectly, by dividing cells.

Recently there has been renewed interest in the question of whether normal epidermal cells actively leave the basal layer or whether they are removed passively as a result of forces emanating from the interaction of cells in the basal layer. There is no doubt that individual epidermal cells have the capacity to move, and they do so actively during wound healing *in vivo* (Winter, 1972) and in many different circumstances *in vitro* (Mitrani & Marks, 1978; Stenn, Madri & Roll, 1979). The mechanisms responsible for cell displacement upwards from the basal layer during normal cell replacement have yet to be elucidated. The simplistic concept of cells moving out of the basal layer as a result of population pressure generated by dividing cells (passive migration) arose many years ago (Bizozzero, 1888) and was reintroduced with supporting evidence by Leblond, Gruelich & Pereira (1964). Etoh, Taguchi & Tabachnick (1975) have challenged this concept by reporting experiments in which beta irradiation inhibited cell division in mouse epidermis but did not slow the rate of transit of irradiated labelled cells from the basal to the granular layer.

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Because of the relevance that cell migration may have in epidermal homeostasis, and the importance that has been given to the work of Etoh *et al.* (1975) in supporting the active displacement concept, we have reexamined the extent to which this concept can be supported by the experimental data. We present evidence which suggests that the migration that occurred in the absence of cell division (Etoh *et al.*, 1975) can still be explained as a passive process in which migration upwards is the result of a population pressure generated by mitosis.

METHODS

In order to simulate Etoh *et al.*'s experiments we have used our computer model of the epidermis (Mitrani, 1976, 1978, 1981). A brief description of some relevant aspects of the model is given in the Appendix. The rules that determine the behaviour of cells are presented here. The three-dimensional model takes into consideration the essential structural features of the epidermis. Normally mitosis occurs only in the basal layer. Entry into mitosis (S phase) is determined by a probability function which is time independent (Smith & Martin, 1983). As cells leave the basal layer (either passively or actively) they progress to the higher layers, becoming flattened and each covering about 16 to 30 basal cells. Maturation and eventual death is time and position dependent (Mitrani, 1981). The phase G1 is subdivided into G1a and G1b. It is assumed that only cells in G1b can leave the basal layer. When a cell enters mitosis, both daughter cells remain basal. Other cells are not always available to be detached and thus competition for space in the basal layer may bring about considerable forces between the cells (Bullough & Mitrani, 1976; Mitrani, 1978). Detachment during G1b is based on viscosity and is quantified according to the viscosity constant, ck. Detachment is therefore pressure and time dependent (Van der Leun *et al.*, 1974).

Essentially two groups of computer experiments were performed (see below). For both groups, the experimental conditions were simulated in the computer on the basis of the kinetic data reported for normal dorsal mouse epidermis by Etoh *et al.* (1975). The only difference between the two groups of computer experiments was that for the first group it was assumed that basal cells actively migrate from the basal layer at a rate equal to the rate of cell production. After irradiation cells were allowed to continue migrating at the same rate. For the second group of experiments, distal cell migration was assumed to be a passive process and therefore cells would be displaced from the basal layer according to the forces acting upon the basal cells. Once mitosis was stopped in the computer experiments, the factor which determined whether a cell would leave the basal layer would be the prevailing configuration and the forces acting on the grip of the cell at the given time (see Appendix).

RESULTS

Active migration, experiments in machina

The first group of computer experiments considers the alternative as stated by Etoh's group and assumes that basal cells actively migrate from the basal layer in normal situations. The onset of migration is determined by a probability function P (L), the probability of migration per cell per hour. This probability is assumed to be equal to the probability of mitosis, to be intrinsic to each cell and not to vary with time.

Figs 1 and 2 show the results for the active migration hypothesis representing conditions reported by Etoh's group. Figs 1 and 2 show that there is some agreement between the results *in machina* and the *in vivo* results (see legends for details). However it can be seen that the kinetic

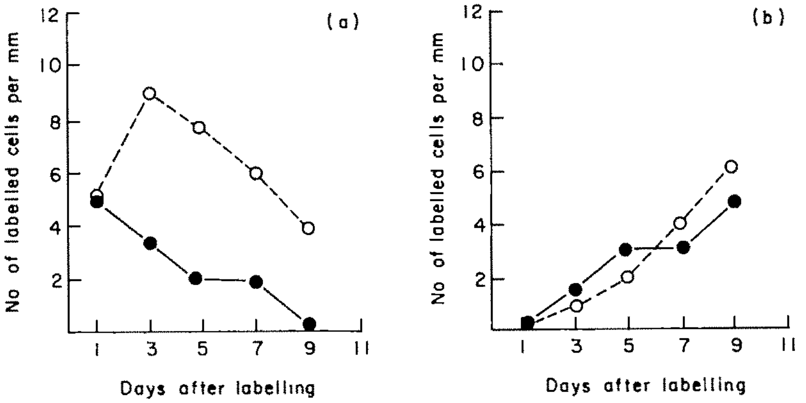

FIGURE 1. Active migration experiments *in machina* (simulated). Changes in labelled cell number of the basal (a) and spinous granular (b) layers in non-irradiated (○) and 'irradiated' epidermis (●). Mitotic rate $= 0\cdot004$ cell/h/cell, $T_{G1a} = 13$ h, $T_S + T_{G2} + T_M = 13$ h.

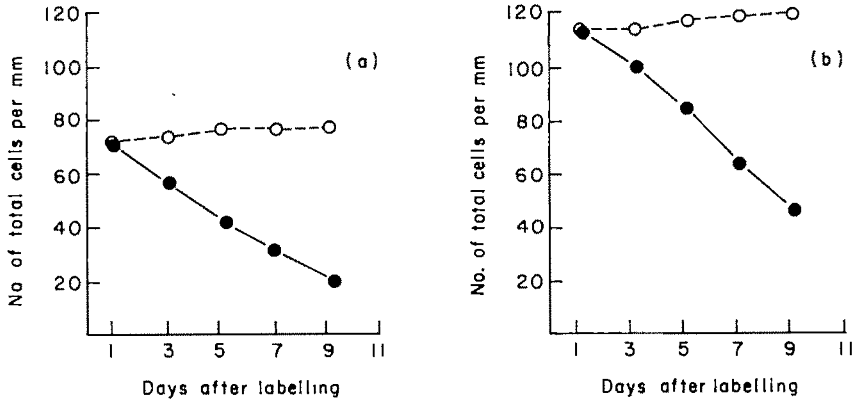

FIGURE 2. Active migration experiments *in machina* (simulated). Rate of depopulation in basal (a) and spinous granular layers (b) after 'irradiation'. Non-irradiated epidermis (○), epidermis 'irradiated' 10 h after labelling (●). Same experiments as those described in Fig. 1.

data responsible for the results shown in Fig. 1 will yield a basal depopulation value of approximately 70% by day 9 if migration were to be active [Fig. 2a (●)]. This high depopulation value is not in agreement with experimental data (compare with Fig. 2a of Etoh *et al.*, 1975). The conclusion must be that active migration under such circumstances is improbable.

Passive migration, experiments in machina

Three conditions had to be met by the simulated experiments on passive migration. First, that the reported experimental mitotic rate, given the appropriate viscosity conditions can produce sufficient overcrowding in the basal layer; second, that those viscosity conditions will allow migration rates comparable to those reported by Etoh's group (1975) when mitoses are normally occurring, and third, that having satisfied conditions one and two, migration rates comparable to those reported [Figs 1b (●) and 6b (●), Etoh *et al.*, 1975] could be obtained once mitosis had been stopped. For the experiments on passive migration it was assumed that cells are formed with a strong attachment to the basal substrate and are gradually detached under the effect of the external forces caused by mitosis (Fm) and overcrowding (Fn). Good agreement with experimental findings was found for the situations described in Figs 3 and 4. Comparatively low values of non-basal labelled cells were found however in those experiments which simulated the effects of irradiation 18 h after labelling [Fig. 5b (●)].

DISCUSSION

Potten's group (Potten & Allen, 1975; Potten, 1980, 1981) has often questioned the validity of the passive migration hypothesis and has attempted to design experiments which might help to

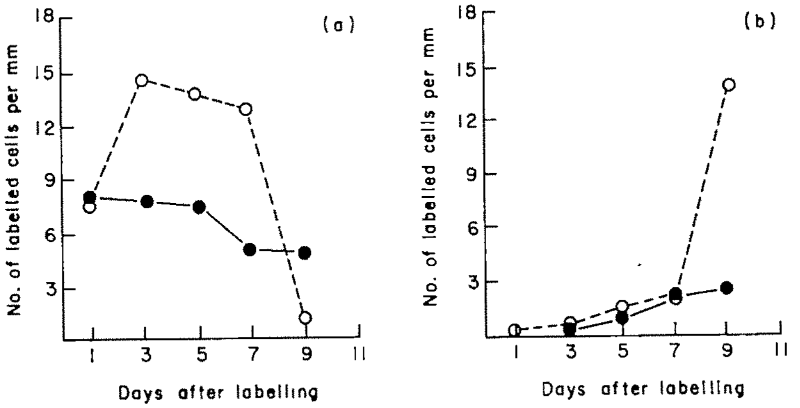

FIGURE 3. Passive migration experiments *in machina* (simulated) Changes in labelled cell number in the basal (a) and spinous granular (b) layers in non-irradiated (○) and epidermis 'irradiated' 10 h after labelling (●). Mitotic rates: 0·0044 + $G_{1a} = 13$ h, $T_S + T_{G2} + T_M = 13$ h. Basal grip is not renewed unless the cells re-enter the S phase.

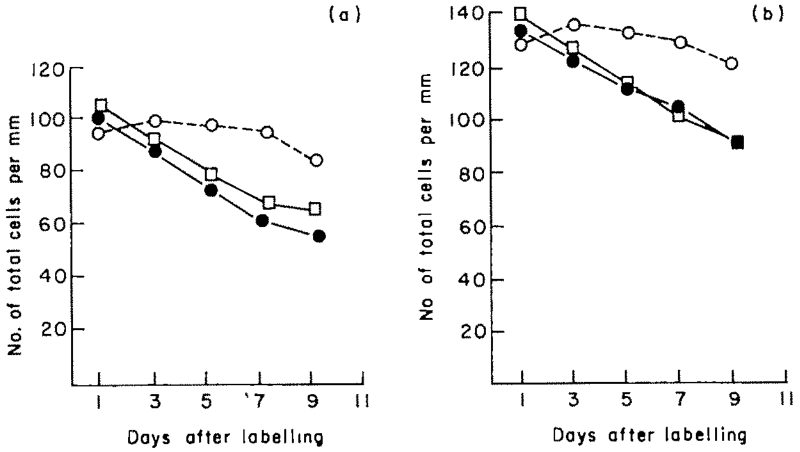

FIGURE 4. Passive migration experiments *in machina* (simulated) Rate of depopulation in basal (a) and spinous granular layers (b) after 'irradiation'. Non-irradiated epidermis (○), epidermis 'irradiated' 10 h after labelling (●), epidermis 'irradiated' 18 h after labelling (□). Same experiments as those described in Fig. 3.

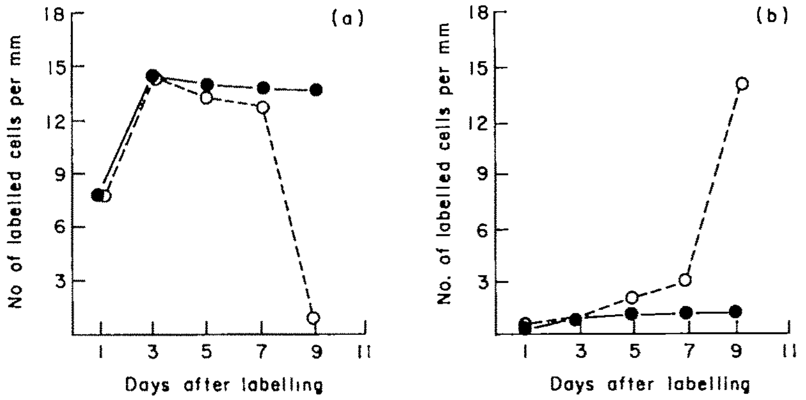

FIGURE 5. Passive migration experiments *in machina* (simulated). Changes in labelled cell number in the basal (A) and spinous-granular (B) layers in non-irradiated (○) and epidermis 'irradiated' 18 h after labelling (●). Same experiments as those described in Fig. 3 but irradiation was simulated to happen 18 h after labelling.

clarify this kinetic aspect. Potten & Allen (1975) have claimed that mild disruption of epidermis by tape stripping results in rapid movement of cells into the suprabasal layer (after about 12 h) and that this movement *precedes* mitotic activity. However, it is difficult to know how this conclusion was obtained since no data on migration rates was given. Yet from the mitotic activity data it is clear that although the peak (burst) of mitotic activity occurred by about 18 h, by 12 h after stripping there was at least a twofold increase in mitotic activity which would be clearly sufficient to account for the migration 'rates', described in their study.

If in normal mammalian epidermis, cell migration distally were to be an active process there could be two alternatives. The first alternative would be that the processes of distal cell migration and the process of mitosis are uncoupled. Stability would require in such a case that the rate of basal cell migration would be close to the rate of basal cell production. We have performed computer simulations to test this alternative (not shown) and found that the stability of such a system would be highly sensitive to small deviations in the value of one rate with respect to the other. It is difficult to think how a biological system, known to be capable of experiencing and coping with variations of some orders of magnitude in rate of cell production, could survive under such strict stability considerations. Clearly the rate of migration and the rate of mitosis must be coupled. The remaining alternative would then be that active basal cell migration would be the driving force. This would imply that a cell leaving the basal layer would in some way trigger another cell into mitosis (Potten, 1980). This alternative is also considered unlikely on a theoretical basis since such a configuration would be unlikely to create a conflict situation in the basal layer. We have presented theoretical evidence which suggests a correlation between an increase in mitotic activity and a decrease in the chance of a new daughter cell finding a place in the basal layer (Bullough & Mitrani, 1976). We have also shown experimentally that as the mitotic rate increases either one or a combination of some of the following events are observed: increase of basal surface area as indicated by an increase in folding of the dermo-epidermal baseline, increased number of cells per unit baseline, and increased number of vertical mitoses (Mitrani, 1978).

The first group of computer experiments assumed that migration of basal cells in Etoh *et al.*'s (1975) irradiation experiments is an active process. These computer experiments did not produce results that were in agreement with Etoh's own experimental data. The rate of basal cell migration which had to be assumed in order to be consistent with the kinetics of the process examined (cell production equal to cell loss, for steady state conditions) generated a depopulation value far higher than the one observed experimentally. On the basis of the simulated experiments one would have to conclude that active migration was not the driving force or that the rate of basal cell migration after irradiation was not the same as the rate of cell migration prior to irradiation. If the latter case were to be true Etoh *et al.*'s reported experiments (1975) would be totally inconclusive.

The second group of computer experiments which assumed instead that basal cell migration is a passive process, yielded results which were comparable to those obtained experimentally by Etoh's group (1975). However when irradiation was assumed to happen 18 h after labelling these simulations produced low migration rates when compared with the experimental data. We believe that the higher migration values obtained in the experimental situation might have been due to the fact that by 18 h after labelling, not all labelled cells may have entered mitosis by the time irradiation took place (see Fig. 6 of Etoh *et al.*, 1975).

Clearly the simulations under the passive migration hypothesis show sufficient similarity with the experimental findings of Etoh *et al.* (1975) so as not to have to discard cell division as the driving force in the process of distal cell migration from the basal layer in normal mammalian epidermis.

On the basis of the available data and the experiments examined above we believe that the evidence favours the concept that in normal steady state epidermal populations, cells are passively removed from the basal layer as a result of forces generated directly or indirectly by dividing cells.

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APPENDIX

The epidermis is essentially represented by a three-dimensional matrix TIM (I, J, Z). I and J are variables which represent the X and Y coordinates on the horizontal plane, and the variable Z represents the vertical components. Each cell occupies a position (I, J, Z) in space. The matrix K (I, J, Z) contains information as to which type of cells are present (see below) and where they are located within the matrix. If $K (I, J, Z) = 0$ this indicates that position I, J, Z is empty, otherwise there is a cell whose type depends on the value of K (I, J, Z). The matrix TIM (I, J, Z) stores the age of each cell.

The initial cell configuration is determined arbitrarily at the beginning of the simulation (time zero). A single cell is sufficient to generate the whole epidermis after a sufficient number of simulation cycles. Each simulation cycle entails incrementing the simulation time by one and increasing accordingly the age of each cell by one simulated hour. During each cycle the program scans the whole matrices and whenever it finds a cell, according to its type, age, position and according to the rules described above (see Methods); the program decides for instance, whether that particular cell is on the basal layer and if so, according to its age, whether it is ready to enter the S phase. If that particular cell is ready to enter the S phase the computer generates a random number according to the probability function described above, and depending on the number generated, it determines whether the cell should enter the mitotic phase. If a cell in (I, J, Z) is ready to divide, the computer has to choose a position to allocate the new daughter cell. It thus examines those surrounding positions which constitute the neighbourhood of the dividing cell in order to determine whether any of those positions are empty (i.e. if for instance $K (I, J+1, Z) = 0$), then the program will make $K (I, J+1, Z) = 1$ and thus creates a new cell with $TIM (I, J+1, Z) = 0$. Failing this the program determines whether any of the neighbouring cells of the dividing cell is in G1b (i.e. if TIM (I + 1, J, Z) for instance, > G1a) in which case the cell in position I + 1, J, Z will be extruded from the basal layer and relocated in a position immediately above it, i.e. $TIM (I+1, J, Z+1) = TIM (I+1, J, Z)$ and $K (I+1, J, Z+1) = 1$, $TIM (I+1, J, Z) = 0$. One daughter cell will occupy the position of the mother cell i.e. $TIM (I, J, Z) = 0$. The other cell will be allocated to the empty position found or to the position that has been made empty when one of the neighbour cells in G1b was moved upwards. If the situation arises that no position in the neighbourhood is available and no neighbour cell can be extruded, then the computer proceeds to create extra spaces between the cells in the basal layer (see Mitrani, 1981) and thus contributes to the crowding of the layer. Basal cells are continuously being submitted to two types of forces: one local Fm resulting from the nearby mitosis, and another global Fn which may result from an excess number of cells in the basal layer. Consequently the model incorporates folding of the basal layer which may result from overcrowding, and thus comprises a basal layer which can expand and contract dynamically. The time needed to release a new daughter cell should be equal to $TG1a + ck/Fn$. If n9 is the number of basal cells per unit length in normal conditions and n8 the number of basal cells per unit length when the basal layer is folded, then the force Fn will be calculated as $Fn = (n8 - n9)/n9$. It is also assumed that the detachment fraction experienced by G1b cells due to Fm will be equal to $1/ck$.

The irradiation experiments were simulated by introducing an additional rule: after the simulation had evolved for a sufficient number of cycles to achieve a steady state, cells were not allowed to divide further. All other rules remained the same. The ^{3}H-labelling of cells was simulated by creating an additional stage K(I, J, Z) = 2. This indicated that a cell in position I, J, Z was thymidine 'labelled'. All cells were created with K (I, J, Z) = 1. At a particular time in the simulation only those cells which happened to be in the S phase were altered so that their K status would become equal to 2. Finally, results were recorded by examining at a particular time each cell of the matrix and recording its status, age and position so that the graphs presented above were obtained in approximately the same way as they would have been in real life, e.g. by counting and recording what percentage of the cells were labelled (K status = 2) in the basal layer at a given time. By examining consecutive cycles it was possible to record for instance when 'labelled' cells started to appear in suprabasal layers (i.e. cells with K values = 2 and Z values $\neq$ 1) and thus to determine the rate of migration of 'labelled' cells from the basal layer.

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Epidermolysis bullosa simplex blister fluid induces an intra-epidermal blister in cultured normal skin

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SUMMARY

Organ cultures of normal human skin incubated with blister fluid from a patient with epidermolysis bullosa simplex (EBS) produced a clean intra-epidermal blister with histology similar to that of an EBS blister.

Epidermolysis bullosa simplex (EBS) is an autosomal dominant, non-scarring, localized epidermolysis. Blisters are induced by trivial trauma and the frequency of blistering increases with increasing ambient temperature.

Two hypotheses have been proposed for the pathogenesis of this disease, but they are not entirely convincing. The first is the activation theory of cytolytic enzyme(s) proposed by Pearson (1971), and the second is the existence of a mutant, temperature-sensitive structural protein proposed by Gedde-Dahl (1977). However, the precise mechanism involved in blister formation is unknown.

METHODS

Patient

A 28-year-old woman was known to have had blisters on her hand from 2 days after birth. She continued to develop new bullae and erosions at sites of friction and trauma such as neck, axillae, hands and feet throughout her infancy and childhood.The frequency of blistering increased with the increasing ambient temperature and, as a result, blisters were easily induced during the summer months. At the age of 14, blister formation gradually decreased and became localized to the hands, lower legs and feet. The lesions healed without scarring or milia formation. On admission to our hospital, she had blisters on the lower legs, feet and hands. There was no

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history of blistering in the mucous membrane. Her family history for similar skin findings was negative. Light microscopy of her skin specimen revealed a clean intra-epidermal blister without inflammatory infiltration. PAS-positive basement membrane was seen on the floor of the blisters. Silver-positive materials were also shown on the floor of blisters (Fig. 1a). Electron microscopy revealed marked cytolysis of basal cells. The basement membrane, dermis, basal lamina and anchoring fibrils were almost all intact.

Blister fluid

Blister fluids were obtained from fresh blisters arising from the skin of the patient with EBS, from a patient with burns and from a healthy person with suction blisters. Blister fluids were immediately centrifuged at 3000 r.p.m. for 10 min at 4°C and the resultant supernatants were used for organ culture.

Organ culture

The organ culture was carried out according to the method described by Morioka, Naito & Ogawa (1981). Normal human skin obtained during surgery was used. Slices of this normal skin were cultured in various culture media for 12, 24, 48 and 72 hours. Each culture medium contained blister fluid from either the EBS patient, the patient with burns or the suction blister obtained from the normal healthy person. After the stated times, the skin specimens were stained by routine methods (haematoxylin and eosin, PAS, and silver stains).

RESULTS

No remarkable histological findings were observed in the skin explant cultured with blister fluids from the burned patient or the suction blister. On the other hand, the skin explant cultured with blister fluid from the patient with EBS showed a clear intra-epidermal blister formation similar to the histology of EBS *in vivo*. The initial intra-epidermal blister formation started at 24 hours and increased throughout the increasing culture period, with a clean intra-epidermal blister formation being observed after culturing for 72 hours. PAS-positive basement membrane was observed on the floor of the bulla. As shown in Fig. 1b, silver-positive materials were also clearly shown on the floor of the blister. There was no visible cell damage, such as vacuolation and dyskeratosis, after culturing for 72 hours.

DISCUSSION

The pathogenic mechanism involved in blister formation in EBS is at present unknown. In this present study, the pathogenesis of EBS was investigated by culturing normal healthy skin with blister fluid from a patient with EBS. As seen in Fig. 1b, blister fluid induced a clean intra-epidermal blister formation in normal healthy skin similar to that of the histology of EBS *in vivo*. This finding suggests that the blister fluid from a patient with EBS contains some specific factor, such as an enzyme, which induces the intra-epidermal blister, and it also demonstrates that light microscopic features resembling those of EBS *in vivo* can be induced in normal skin without any congenital defects of the structural proteins. We have recently reported that blister fluid from a patient with recessive dystrophic epidermolysis bullosa (RDEB) induced a clean dermal-epidermal separation similar to the histology of RDEB *in vivo* in a normal healthy skin explant culture (Takamori *et al.*, 1983). We also reported that collagenase and neutral protease activities in blister fluid from a patient with RDEB were higher than those

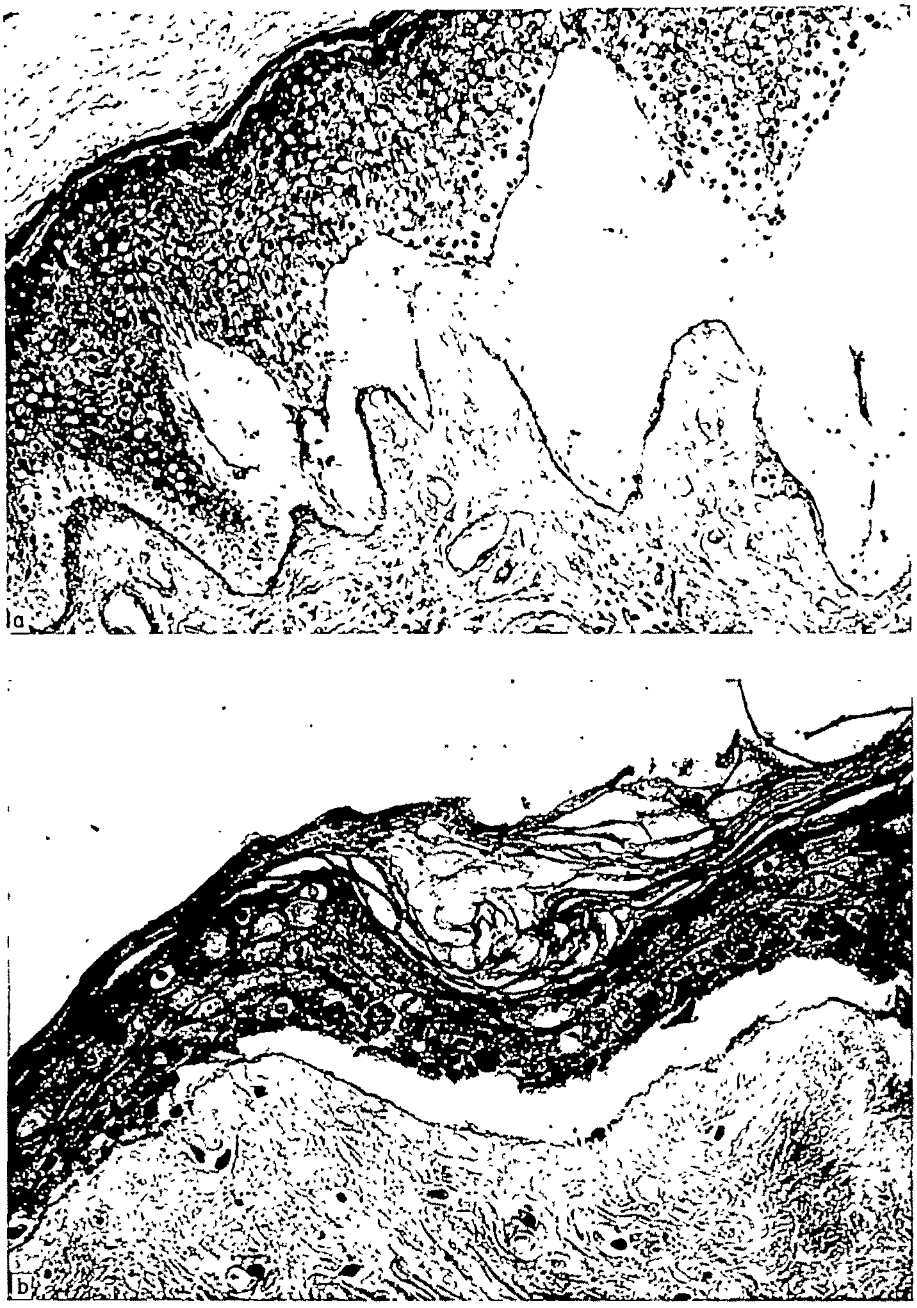

FIGURE 1 (a) Light micrograph of a patient with epidermolysis bullosa simplex. The biopsy was taken 12 h after friction A band of strong silver-positive material is seen on the dermal side of the blister. Silver stain (× 400). (b) Histology of normal human skin explant cultured for 72 h with blister fluid from a patient with epidermolysis bullosa simplex. A clean intra-epidermal separation can be observed. Silver-positive material is seen on the floor of the blister. Silver stain (× 200) found in burns blister fluid. We suggested that blister formation in RDEB can be induced in normal skin with no congenital defects and not only collagenase but also neutral protease may be implicated in the formation of blisters in RDEB skin (Takamori *et al.*, 1983). The identification of the specific factor in the EBS blister fluid which induced the intra-epidermal blister formation in organ culture is now in progress. Further experiments with electron microscopic studies using cultured skin are now needed.

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A more reliable photometric technique for the measurement of scalp sebum excretion

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SUMMARY

The objective assessment of antiseborrhoeic therapy for the scalp requires a reliable technique for the measurement of sebaceous gland activity. Previously published methods were tried but found to be unreliable. This paper describes a photometric technique which gave repeatable results and demonstrated a reduction of sebaceous gland activity on the scalp with both oral isotretinoin and topical 11α-hydroxyprogesterone. The accuracy of the method was achieved by careful preparation of the scalp but was affected by recent shampooing.

Sebaceous gland activity of the forehead has been studied extensively and several reliable methods for its measurement have been described (Strauss & Pochi, 1961; Cunliffe & Shuster, 1969; Cunliffe, Kearney & Simpson, 1980). Sebaceous gland activity of the scalp has received less attention although two methods have been described for its measurement *in vivo* (Eberhardt & Kuhn-Bussius, 1975; Saint-Leger & Leveque, 1982). While conducting a pilot study for a clinical trial of an antiseborrhoeic preparation for greasy scalps the available methods were tried but found to be imprecise and unreliable. The present study was performed to develop a reproducible *in vivo* assay for the measurement of sebaceous gland activity in human scalp skin. To assess the usefulness of the method in clinical trials, two short preliminary studies were performed: (a) with oral 13-*cis*-retinoic acid (isotretinoin) and (b) with topical 11α-hydroxyprogesterone (11OHP).

METHODS

Measurements were made with a Lipometre® (Gras, de Rigal & Leveque, 1979). This machine is a fixed wavelength spectrometer which measures the increase in light transmission through a ground sapphire plate when the surface is coated with lipid. Six-mm circular sampling plates were used.

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Sixty adult volunteers (twenty-five male, thirty-five female; aged 16–28 years) were used in the studies to derive the optimum method. Nine adult volunteers (four male, five female) were used in the clinical trial with topical 11OHP. Five adult males with rosacea were used in the clinical trial with isotretinoin.

All measurements were made at two sites lying symmetrically over the mid-parietal area of the scalp. Two values were derived; casual level (CL) and sebum excretion rate (SER). Casual level refers to the amount of sebum measured at any time irrespective of prior preparation of the scalp. SER refers to a timed collection of sebum after prior preparation of the sampling site. In both instances, four successive samplings were performed, as the sum of these measurements has been shown (on the forehead) to correlate well with the amount of lipid collected by the gravimetric method (Cunliffe *et al.*, 1980). All samplings were performed between 24 and 48 h after washing the hair.

Group 1

Fourteen volunteers took part in this study in which both CL and SER were measured. Sampling sites were prepared by clipping the hairs close to the scalp over a circular area 1-cm diameter. CL was measured at the prepared site by four successive samplings of 15 seconds each, following which adsorbent tissue was firmly held against the scalp for a further 30 seconds. Hair was kept away from the sampling sites by adhesive tape and only cold drinks were permitted. After 3 h the sites were resampled a further four times to give a value for SER. This method is similar to that used by Saint-Leger & Leveque (1982).

Group 2

Forty-six volunteers took part in this study in which SER alone was measured. Sampling sites were prepared by clipping the hair close to the scalp as in group 1 but in this group the sampling sites were shaved with a scalpel blade so that stubble was not palpable. The sampling sites were then 'blotted' with adsorbent tissue (as in group 1) and the sampling plates applied. If more than 5 μg per cm^2 lipid was present the sites were 'blotted' again and resampled. At this point the tissue paper sheets showed the characteristic 'follicular pattern' of pilosebaceous openings. The time was noted and the sites resampled 3 h later to give a value for SER.

11α-hydroxyprogesterone

Five females (18–25 years) and four males (20–30 years) were supplied with a 0·5% solution of 11OHP in ethanol/water/sodium lauryl sulphate (70:29:1) and asked to apply 15 ml to the entire scalp twice daily. SER was measured by the method for group 2 above, before and after 1 month of treatment.

Isotretinoin

Five adult males (30–46 years) with rosacea were given isotretinoin (0·5 mg kg^{-1} day^{-1}) and SER was measured by the method for group 2 above, twice before and once after 1 month of treatment.

RESULTS

Group 1

The relationship between measurements from the left and right sides of the scalp is shown in Fig. 1 (CL) and Fig. 2. (SER). The former shows a significant correlation ($r = 0{\cdot}692$, $P < 0{\cdot}05$) but the latter fails to reach statistical significance.

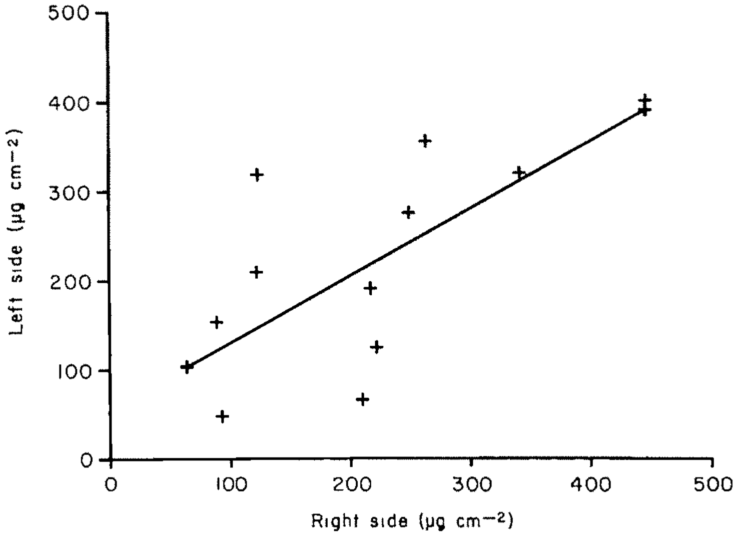

FIGURE 1. Comparison of casual level measurements from left and right sides of the scalp in fourteen volunteers from group 1. Correlation coefficient $r = 0{\cdot}692$ ($P < 0{\cdot}05$).

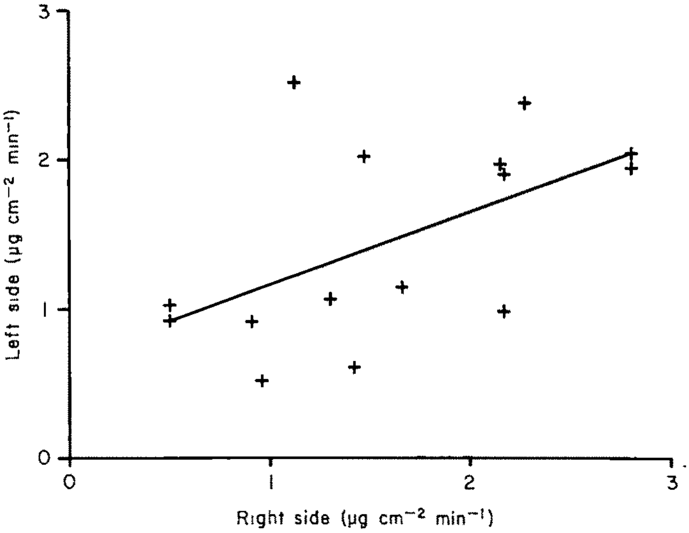

FIGURE 2. Comparison of sebum excretion rate values from left and right sides of the scalp in thirteen volunteers from group 1. Correlation coefficient $r = 0{\cdot}476$ (P: ns).

Group 2

The relationship between SER measured on the left and right sides of the scalp is shown in Fig. 3 and demonstrates a very high correlation ($r = 0{\cdot}92$, $P < 0{\cdot}001$).

Fig. 4 shows the effect of 11OHP and isotretinoin on the sebaceous gland activity of scalp. Statistical analysis (Mann-Whitney U test) showed a significant reduction in SER for the females (but not the males) using 11OHP ($P < 0{\cdot}05$) and a highly significant reduction in SER

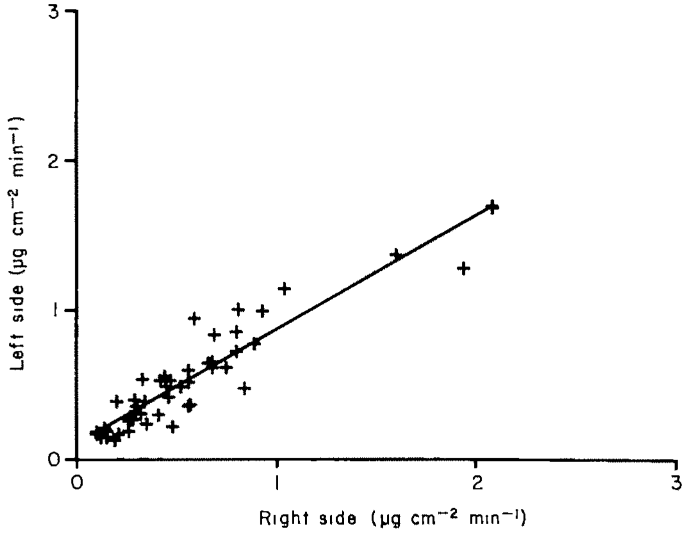

FIGURE 3. Comparison of sebum excretion rate values from left and right sides of the scalp in forty-six volunteers from group 2. Correlation coefficient $r = 0·921$ ($P < 0·001$)

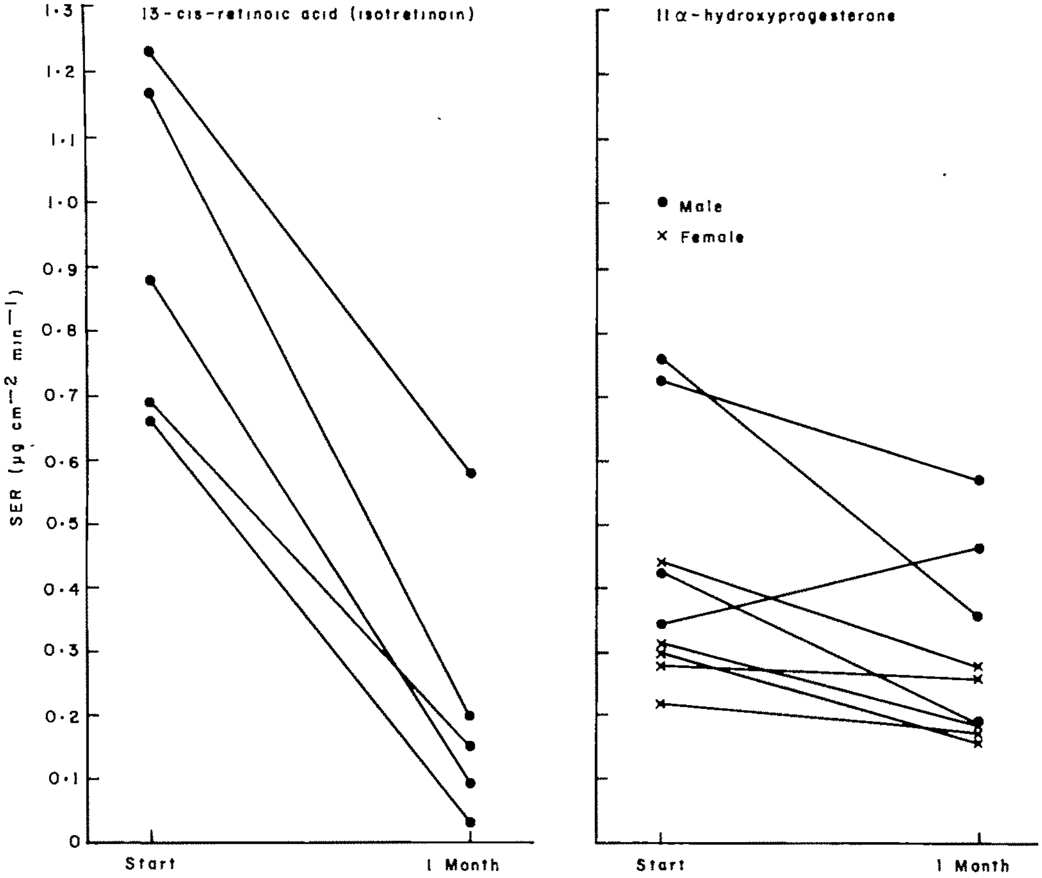

FIGURE 4. The effect of oral isotretinoin (0·1 mg/kg/day) and topical 11α-hydroxyprogesterone (0·5%) on sebum excretion rate of scalp. Each point represents the mean of samplings from the left and right sides of the scalp. The 'start' values in the isotretinoin group represent the mean of two sets of observations.

for the males taking isotretinoin ($P < 0{\cdot}001$). Analysis of the squalene content of sebum eluted from individual hairs showed an overall reduction of 60% in the latter group.

Effect due to shampoo

Ten volunteers were assessed at weekly intervals on three occasions and asked to shampoo their hair according to three schedules: (a) less than 24 h, (b) 24–48 h, (c) 48–72 h, before measurement. The schedules were assigned randomly. The results are shown in Table 1. Analysis of variance showed a significant reduction when the hair was shampooed less than 24 h before measurement ($P < 0{\cdot}01$). There was no significant difference between the values obtained when hair was shampooed 24–48 h or 48–72 h before measurement.

DISCUSSION

Due to the inherent symmetry of the integument, studies used to assess and validate methods for the measurement of SER have compared the left and right sides of the body (Cunliffe & Shuster, 1969; Cunliffe, Kearney & Simpson, 1980). Results from the present study indicate that preparation of the scalp is of paramount importance. Methods for the measurement of SER on the scalp have been described before, but all except one (Saint-Leger & Leveque, 1982) have sampled lipid overlying hair. The removal of hair at the sampling sites makes possible the close approximation of sampler and scalp. The results from group 1 indicate that merely clipping the hair level with the scalp (the method used by Saint-Leger & Leveque, 1982) yields a poor left/right correlation when the 3-h SER is measured. This error is probably due to poor contact with scalp from scales, epithelial debris and bristles. When the hairs were shaved level with the scalp after clipping a very high left/right correlation was obtained (Fig. 3). The latter method was therefore the method of choice and was used in the clinical trials. The casual level could not be measured by this method as shaving removed a variable amount of surface lipid.

The two clinical trials performed indicate that the method responds to a positive control by

TABLE 1. The effect of shampooing on scalp SER

	Less than 24 h	24–48 h	48–72 h
	0·21	0·46	0·44
	0·13	0·3	0·33
	0·13	0·77	0·61
	0·47	0·69	0·77
	0·48	0·77	0·51
	0·47	0·65	0·81
	0·57	0·86	0·75
	0·57	0·78	0·86
	0·41	0·6	0·62
	0·26	0·45	0·47
Mean	0·36**	0·63	0·62
s.d.	0·17	0·18	0·18

** $P < 0{\cdot}01$ by analysis of variance.

demonstrating a reduction in SER. The response of females to topical 11OHP confirmed the findings of Tamm *et al.* (1982) who measured SER on unclipped scalp, a method which is dependent on both SER and refatting kinetics.

The method is unaffected by shampooing provided that at least 24 h are allowed to elapse after the last shampoo. This effect is probably due to removal of sebum from a follicular reservoir which then needs to be refilled. This would accord with the conclusions of Eberhardt (1976) and the findings of Saint-Leger & Leveque (1982) that scalp may contain a large 'follicular reservoir' of sebum, the size of which may vary independently from changes in SER. A similar situation has been demonstrated on forehead skin by Downing, Stranieri & Strauss (1982). These workers used bentonite clay to absorb skin surface lipid and found that a sustainable level of sebum secretion was not reached until 12 h after a degreasing procedure. The 12-h difference in time scale may be explained by differences in both sampling site and method. However, it is important to give detailed instructions to patients because many feel that they should wash their hair before attending.

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Precipitins to dietary proteins in atopic eczema

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SUMMARY

Precipitating antibodies to foods have been assayed in three groups of patients with atopy. Forty-five per cent of patients with atopic eczema and IgE-mediated food allergy had precipitins to foods in their serum compared with only 15% of patients with atopic eczema without evidence of food allergy, and 16% of patients with atopic asthma and/or rhinitis. It is likely that this results from increased intestinal permeability in the group with eczema and food allergy.

The pathogenesis of the eczema component of atopy remains obscure. Lesions in the skin of atopic eczema patients are not typical of IgE-mediated hypersensitivity, since in acute eczema there is no increase in the number of mast cells and no evidence of mast cell degranulation or eosinophil infiltration. In eczema the skin infiltrate consists of mononuclear cells and the main pathological changes occur in the epidermis. This contrasts with the other atopic manifestations, which include asthma, rhinitis and conjunctivitis, which are typical of IgE-mediated hypersensitivity. Nevertheless, in some patients there appears to be a relationship between allergy to food and atopic eczema, in that the skin lesions can be ameliorated by a diet excluding eggs and cows' milk (Atherton *et al.*, 1978).

We have previously reported that many atopic eczema patients have high titres of IgE class antibodies to foods (Barnetson, Merrett & Ferguson, 1981). We have further examined immune responses to foods in atopic eczema by using a simple microprecipitin technique to detect non-IgE food antibodies. Patients with atopic eczema have been contrasted with a group of atopic patients with respiratory allergy alone (asthma and/or rhinitis).

METHODS

Patients

Three groups of adult patients were studied.

(1) Atopic eczema with 'food allergy'. In these patients the presence of atopic eczema was accompanied by either a history of angio-oedema following ingestion of a food (egg, cows' milk or fish) or else the patients avoided ingestion of these foods because of dislike or because the food

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was not part of their normal day-to-day diet; each had a strongly positive (Pharmacia Phadebas RAST score 3 or 4) titre of IgE antibody to one or more of these foods. This group comprised thirty-eight patients, twenty with a history of angio-oedema and eighteen others.

(2) Atopic eczema 'without food allergy'. These twenty patients had no history of clinical reaction to foods, and did not have strongly positive RASTs to foods.

(3) Respiratory allergy. These patients had asthma and/or rhinitis, and were of comparable age and sex to the atopic eczema patients; none of them had atopic eczema or a history of clinical food allergy. There were eight patients with asthma and rhinitis, six with asthma and eleven with rhinitis, totalling twenty-five in all.

Precipitin tests

Precipitating antibodies were detected in serum collected from the above patients by using a modification of Crowles' micro-gel diffusion technique (Ferguson & Carswell, 1972). Samples were coded and tests performed in duplicate. Immunodiffusion occurred in a 1-mm thick layer of agar on a microscope slide; patients' serum and antigen were placed in holes in a perspex template lying on top of the agar. After 48 hours' incubation, slides were washed and precipitin lines were stained with thiazine red. The antigens used were prepared as previously described (Ferguson & Carswell, 1972): 1 in 2 and 1 in 10 dilution of skimmed cows' milk in phosphate-buffered saline; 1 in 50 dilution of filtered egg yolk and egg white in PBS; 1 in 10 dilution of chicken serum and bovine (calf) serum; saline extracts of pork and codfish prepared by freezing, thawing and filtering a homogenate of antigen in PBS, the concentration adjusted to 2–4 μg/ml; saline extract of wheat gluten. Significant differences between groups were calculated by using the chi-square test.

RESULTS

The results in the three groups of patients are summarized in Table 1. Forty-five per cent of the thirty-eight patients with eczema and food allergy had precipitating antibodies to one or more of the food antigens studied. There was no correlation between the presence of precipitating antibody to a particular food and either a history of allergy to that food, or of IgE antibodies to the food concerned. In contrast, the twenty patients with atopic eczema but without evidence of food allergy had a low prevalence of precipitating antibodies, similar to that in the asthma/rhinitis control group. The differences between the group with atopic eczema and food allergy, and the other two groups were significant ($P < 0\cdot01$ in each case).

TABLE 1. Serum precipitins in three groups of patients eczema with food allergy, eczema without food allergy and asthma and/or rhinitis

	Total no.	No with precipitating antibodies	Milk	Egg	Fish	Wheat	Chicken	Beef	Pork	No. without precipitating antibodies
Eczema + food allergy	38	17 (45%)	3	9	6	2	1	2	5	19 (55%)
Eczema alone	20	3 (15%)	1	0	1	0	0	0	2	17 (85%)
Asthma/rhinitis	25	4 (16%)	1	0	0	1	1	1	1	21 (84%)

DISCUSSION

The finding of precipitating antibodies to foods in patients with atopic eczema is of considerable interest and shows that the immunological abnormality in these patients extends beyond the IgE class of antibodies. Serum antibodies to many foods are present in patients with coeliac disease and villous atrophy in jejunal biopsies, but this is not the explanation for serum food antibodies in atopic eczema as the majority of these patients have completely normal jejunal biopsy morphology (Braathen *et al.*, 1980; Barnetson *et al.*, 1981). By using small marker molecules, some patients with atopic eczema can be demonstrated to have abnormal small bowel permeability (Jackson *et al.*, 1981), although the size of the test molecules used for such signs of permeability is considerably lower than would be relevant to an immunogen or food antigen. Nevertheless abnormal permeability of the gut in atopic eczema could be the explanation for the presence in serum of precipitating antibodies induced by exposure of the systemic immune apparatus to abnormally large amounts of food antigen. At present, techniques suitable for studying passive permeability of the gut to antigens are not developed.

The immunoglobulin class and subclass of the precipitating antibodies we demonstrated are currently being studied in our laboratories. One possibility is that they are IgG4 antibodies, for it has been shown that atopic eczema patients have high concentrations of these antibodies (Shakib *et al.*, 1977; Barnetson & Merrett, 1983), which are potentially relevant to mast cell degranulation. Whether antibodies of this subclass, or other classes of precipitating antibody have a direct pathogenetic role in the skin lesions of atopic eczema, perhaps via immune complex deposition, remains to be established.

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Furthermore, patients were questioned about the additional use of systemic drugs which might also inhibit or stimulate hair growth.

RESULTS

Most patients in the PUVA group (fifteen out of twenty-three; 65%) exhibited moderate to marked hypertrichosis (Table 1). In the UV-A category hypertrichosis was observed in only two out of fourteen cases (14%). The difference between the two groups is highly significant ($\chi^2 = 9{\cdot}09$; $P < 0{\cdot}005$). On the whole, PUVA-induced hypertrichosis was more severe than in the UV-A group. Six PUVA-treated patients found the hypertrichosis to be embarrassing. Three of these patients had already been treating the hypertrichosis; one patient had started to shave her face, a second had used resin to remove excessive hairs on the hands and a third had acquired the antiandrogenic contraceptive pill Diane® through a friend. This last patient experienced the hypertrichosis as a serious side-effect, outweighing the beneficial effect of PUVA therapy on her psoriasis. The other patients found the changes in hair growth less distressing than their initial skin problem. Hypertrichosis was most marked on the face and the extremities, less so on the trunk. Excessive hair growth started within 6–12 weeks after the commencement of PUVA therapy.

Age of the patient, hair colour and skin type had no bearing upon the development of hypertrichosis. We had the impression that the absence of hypertrichosis in the PUVA group was related to low cumulative dosage of UV-A (< 180 J/cm^2) or to poor patient compliance (irregularity of treatment). The impact of the C_{max} on hair growth activation could not be satisfactorily assessed since in several PUVA patients C_{max} had not been established. Four patients were using oral contraceptives but the development of hypertrichosis was not related to this use. Five women were regularly using drugs other than oral contraceptives, most of which were not known to cause hypertrichosis. Patient No. 17 was using lithium therapy which has been reported to cause alopecia. Even so, this patient developed a severe hypertrichosis under PUVA therapy. Patient No. 3 was using cimetidine, an antiandrogenic drug which has a beneficial effect on hirsutism. In this case, however, the low cumulative UV-A dose (117 J/cm^2) seems a more likely explanation for the absence of hypertrichosis than the use of cimetidine.

DISCUSSION

PUVA treatment stimulates the keratinocyte in the basal layer of the epidermis. Mitoses are more numerous and hyperkeratosis ensues. UV-A alone does not have such a potent stimulatory effect. Whereas hyperpigmentation is seen after UV-A exposure, hyperkeratosis of the epidermis remains inconspicuous, and this might explain our findings. If PUVA therapy activates the basal keratinocytes this effect probably also occurs in the hair bulb. Small amounts of long wave ultraviolet radiation may penetrate the dermis down to the level of the follicles, thus eliciting a photodynamic effect in the presence of psoralens, resulting in the occurrence of hypertrichosis. This stimulatory effect of PUVA treatment on the hair follicle has been employed for the management of alopecia areata.

It is surprising that hypertrichosis after PUVA therapy has previously only been sporadically reported (Hofmann *et al.*, 1977; Heise *et al.*, 1980). This could be due to the fact that only those patients who had spontaneously complained about abnormal hair growth were recorded as exhibiting hypertrichosis. If all patients are systematically questioned about this side-effect, a much higher proportion of hypertrichosis is revealed. In the pre-PUVA era systemic psoralens

TABLE 1. Hypertrichosis due to photochemotherapy

Diagnosis	Cumulative dose (J/cm^2)	Number of treatments	Maximum serum concentration C_{max} (ng/ml)	Hypertrichosis
PUVA-treated patients				
1. Granuloma annulare	102	18	157	−
2. Psoriasis	115	25	161	+
3 Psoriasis	117	20	158	−
4. Psoriasis	124	21	278	+ +
5. Psoriasis	139	20	130	−
6. Psoriasis	142	21	106	+
7. Psoriasis	155	34	279	+
8. Psoriasis	173	30	59	−
9. Psoriasis	174	23	n.d.	−
10. Psoriasis + vitiligo	176	23	380	+
11. Psoriasis	181	23	192	+
12. Psoriasis	184	21	n.d.	+
13. Psoriasis	190	25	82	+
14. Psoriasis	196	27	109	+
15. Psoriasis	203	25	n.d.	−
16. Psoriasis	229	25	125	+ +
17. Psoriasis	236	33	203	+ +
18. Alopecia areata	237	30	76	+
19 Psoriasis	249	30	26	+
20. Psoriasis	251	26	n.d.	−
21. Urticaria pigmentosa	363	52	n.d.	−
22. Psoriasis	372	39	n.d.	+ +
23. Alopecia areata	407	56	n.d.	+
UVA-treated patients				
1. Atopic eczema	177	21		−
2. Atopic eczema	201	33		−
3. Vitiligo	203	28		−
4. Vitiligo	205	28		−
5. Vitiligo	214	25		+
6. Vitiligo	215	25		−
7. Vitiligo	273	33		−
8. Vitiligo	321	35		−
9. Vitiligo	409	44		−
10. Vitiligo	421	60		+
11. Vitiligo	544	52		−
12. Vitiligo	564	59		−
13. Vitiligo	580	48		−
14. Vitiligo	591	53		−

n.d. = not done.
Hypertrichosis: − absent; + moderate; + + marked.

were used together with natural sunlight in the treatment of vitiligo. Elliot (1959) and Singh & Lal (1967) described four out of twenty-seven and three out of 100 patients respectively, who had been treated with systemic 8-MOP and sunlight and who subsequently developed hypertrichosis.

Although our observations are somewhat subjective, we feel that the findings are genuine. Our data are in line with the known activating effect of PUVA on the epidermal keratinocyte, and since UV-A alone does not have such a potent stimulatory effect it is not surprising that our UVA-treated women developed hypertrichosis only sporadically. These findings are even more striking if one considers that the average cumulative dosage of UV-A was much higher in the UV-A group than in the PUVA group. Moreover, the hypertrichosis was more pronounced in patients in the PUVA group (eleven had developed moderate hypertrichosis and four severe hypertrichosis). In the UV-A group only two cases were seen to have moderate hypertrichosis. We conclude that PUVA therapy carries the risk of the development of hypertrichosis and we feel that patients should be informed about this fact.

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The association of lichen sclerosus et atrophicus and autoimmune-related disease in males

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SUMMARY

We have investigated the presence of autoimmune-related diseases and autoantibodies in twenty-five men with lichen sclerosus et atrophicus (LSA). These patients had a significantly higher incidence of autoimmune-related disorders than a control population and a higher incidence of autoantibodies than would be expected in the normal male population.

It has been reported that female patients with LSA have an increased incidence of both organ-specific antibodies (Goolamali *et al.*, 1974; Harrington & Dunsmore, 1981; Meyrick Thomas *et al.*, 1982) and autoimmune-related diseases (Harrington & Dunsmore, 1981). We have therefore studied male patients with LSA to see whether they have the same tendency to autoimmune-related problems.

METHODS

From the diagnostic indices of St Thomas' Hospital, London and St John's Hospital for Diseases of The Skin, London, the names and addresses of forty male patients with histologically confirmed LSA of the penis (balanitis xerotica obliterans) or of the trunk and limbs, were obtained and the patients were requested by letter to take part in this investigation. Twenty-eight patients replied, and twenty-five agreed to be interviewed, examined and investigated.

The men were all interviewed and examined by one of us (R.H.M.T.), and were questioned about various aspects of their skin disease; in addition past medical and family medical histories were obtained with particular reference to vitiligo, alopecia areata, thyroid disease, pernicious anaemia, liver disease and insulin-dependent diabetes mellitus. The patients' skin was examined, and the patients were also assessed clinically for any evidence of the diseases

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mentioned above. Blood samples were obtained for full blood count, liver and thyroid function tests, blood sugar and tests for anti-thyroid (thyroid cytoplasm, thyroid microsome and thyroglobulin), anti-smooth muscle, anti-nuclear, anti-mitochondrial and anti-gastric parietal cell antibodies.

As a control group, 25 men of matching ages who were new referrals to the dermatology clinic for the removal of minor cutaneous tumours, were also questioned and assessed for personal and family histories of the autoimmune-related diseases already listed.

RESULTS

The mean age of the patients was 43 years (range 18–75 years) and the mean duration of LSA was 7·1 years. In three patients the LSA was confined to the trunk, whilst in the remaining twenty-two, the LSA was confined to the penis; in no patients was LSA present in both genital and extra-genital sites.

The mean age of the twenty-five control males was 44 years (range 18–79 years).

An autoimmune-related disorder was present at the time of study, or had occurred previously, in five (20%) of the patients, (two patients having vitiligo, and three different patients having had alopecia areata) compared with none of the controls. This difference was statistically highly significant ($P<0{\cdot}001$). In all five patients with an autoimmune-related disease the LSA involved only the penis. No patient had a past history of diabetes mellitus, thyroid or liver disease, or pernicious anaemia, and at the time of the study none of the patients had any physical, haematological or biochemical evidence of any of these diseases.

Two patients gave a family history of one of these diseases in a first degree relative (alopecia areata in one, thyrotoxicosis in one). One of the controls gave a history of one of the diseases (diabetes mellitus) in a first degree relative (see Table 1). Nine patients (36%) had a positive autoantibody, anti-smooth muscle antibodies in seven patients and anti-gastric parietal cell antibodies in two patients (tests for anti-intrinsic factor antibodies were not undertaken); the two patients with anti-gastric parietal cell antibodies were both aged more than 60 years.

There was no difference in the duration or severity of LSA in those patients with an autoimmune-related disease or a positive autoantibody compared with the other patients.

TABLE 1. Autoimmune-related disorders

	Patients (n=25)	Patient relatives (n=143)	Controls (n=25)	Control relatives (n=141)
Thyrotoxicosis	—	1	—	—
Hypothyroidism	—	—	—	—
Diabetes mellitus (insulin dependent)	—	—	—	1
Vitiligo	2	—	—	—
Alopecia areata	3	1	—	—
Pernicious anaemia	—	—	—	—
Liver disease	—	—	—	—
	5	2	0	1

DISCUSSION

Wallace (1971) suggested the possibility of an association between LSA and vitiligo, and subsequently Goolamali *et al.* (1974) investigated the autoantibody status of twenty-six patients (twenty-five female, one male) with LSA and found that they had a higher incidence of organ-specific antibodies than a control population (anti-gastric parietal cell antibodies occurring most frequently). Similar findings have been reported in two later studies (Harrington & Dunsmore, 1981; Meyrick Thomas *et al.*, 1982), both of which showed that 74% of female patients with LSA had one or more positive autoantibodies. Harrington & Dunsmore (1981) also reported that female patients with LSA and their relatives have a significantly higher incidence of associated autoimmune-related diseases than a control population.

Although one of the patients studied by Goolamali *et al.* (1974) was male, to our knowledge no investigation of the incidence of autoimmune-related diseases and autoantibodies in male patients with LSA has been reported. Our investigation of twenty-five men with histologically confirmed LSA has shown that autoimmune-related diseases occur with greater frequency in these patients than in a control group, the two diseases accounting for the difference being alopecia areata and vitiligo. The more frequent occurrence of these two disorders in male patients with LSA is confirmed when our patients are compared with the general male population, where the prevalence of vitiligo is 0·36% (Howitz *et al.*, 1977) and of alopecia areata approximately 2·5% (Anderson, 1950). We have not found any other autoimmune-related diseases in any of these patients.

In contrast to women with LSA, our male patients with LSA do not appear to have any strong predisposition to autoimmune thyroid disease and pernicious anaemia; this may reflect the observation that women are affected significantly more often than men by autoimmune thyroid disease (Tunbridge *et al.*, 1974) and pernicious anaemia (Chanarin, 1979), unlike vitiligo and alopecia areata which have a roughly equal sex incidence (Howitz *et al.*, 1977; Anderson, 1950).

The incidence of positive autoantibodies in our patients is greater than would be expected in the general male population (Hawkins *et al.*, 1979), however it is only half the incidence observed in women with LSA (Harrington & Dunsmore, 1981; Meyrick Thomas *et al.*, 1982). This difference probably reflects the generally lower incidence of autoantibodies observed in men compared with women (Hawkins *et al.*, 1979).

In none of the seven patients we have reported in whom anti-smooth muscle antibodies were present was there a personal or family history of liver disease or any abnormality of tests of liver function, and in all seven patients the anti-smooth muscle antibody was of low titre; in contrast to anti-gastric parietal cell, anti-thyroid and anti-nuclear antibodies, anti-smooth muscle antibodies have only a low persistence over 6 years in males (Hawkins *et al.*, 1972). These observations suggest that the finding of anti-smooth muscle antibodies in these patients does not imply a serious risk of future liver disease.

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The results (to 1981) were previously reported in a study of the outcome of allergic contact dermatitis (Keczkes *et al.*, 1982). The present study includes patients with negative patch tests. After also excluding patients with a history of atopic dermatitis or psoriasis and/or significant dermatitis at other sites, 462 patients who were considered to suffer from direct irritant hand dermatitis were left.

In 1983 postal questionnaires were sent out to all 462 patients in which simple questions were asked as to the outcome of their dermatitis; whether it cleared completely, or was continuously or periodically affecting them. Specific questions were also asked: if they changed their job and whether this change resulted in the clearance of their dermatitis. The full-time or part-time nature of the work was also recorded. Two hundred and thirteen replies were received (a compliance rate of 46·1%). Of these 188 are incorporated in this study; the rest were discarded for lack of adequate data.

RESULTS

Of 188 patients who replied 130 (69·2%) still had active (continuous or periodic) dermatitis and only fifty-eight (30·8%) were clear.

Of the housewife/part-time cleaner group of fifty-six patients only fifteen (26·7%) were clear and forty-one (73·3%) still had dermatitis.

Most interesting are the figures on the outcome of dermatitis in those who changed their occupation. Of sixty-four patients who changed their occupation, twenty-one (32·8%) had complete remission of their dermatitis, and of 124 patients who did not, thirty-seven (29·8%) had complete remission. Forty-three (67·2%) of those who changed their occupation and eighty-seven (70·2%) who did not change their occupation still had active dermatitis.

DISCUSSION

We have been interested for some time in the outcome of contact dermatitis of the hands, but found it difficult to evaluate published results since most studies dealt with both allergic and direct irritant dermatitis (Mayer, 1938; Phillip, 1954; Hellier, 1958; Estoppey, 1965; Saynisch, 1968; Peter, 1968; Borelli & Düngemann, 1971; Burrows, 1972; Heinze, 1972; Pürschel & Fürst, 1972; Reichenberger, 1972; Fregert, 1975; Turk, 1975; Breit & Turk, 1976; Harrison, 1979). In our previous paper (Keczkes *et al.*, 1982) we described the persistence of allergic contact sensitivity. We now concentrate on a 10-year follow-up study of direct irritant contact dermatitis of the hands.

The results indicate that the outcome of direct irritant contact dermatitis of the hands is far from favourable. It would appear from our studies that two-thirds of the patients still have active (continuous or intermittent) disease 1–16 years after contracting it, as suggested by some previous observers (Mayer, 1938; Phillip, 1954; Hellier, 1958; Burrows, 1972; Fregert, 1975).

Perhaps the most important of our observations is about the effect of change of work. Whereas some earlier investigators (Gomez-Orbaneja & Barrientes, 1938; Skog & Tottie, 1961; Rajka, 1966; Peter, 1968) thought a change of occupation would lead to earlier remission of hand dermatitis, our results indicate otherwise. Our findings are in keeping with other investigators (Koch & Peters, 1939; Hunziker & Musso, 1960; Neumann, 1966; Forstrom, Pirila & Huju, 1969; Fregert, 1975; Cronin, 1980).

Whilst we agree that a change of work, when allergic sensitivity exists, is beneficial, we doubt if change of work (and rehabilitation with all the costs it involves) gives any lasting benefit in direct irritant contact dermatitis of the hands.

The hazards and bias of postal surveys are well known, especially when conclusions have to be drawn from less than half the original pool. However many British cities have a less stable population than Hull, so it may be that this proportion of replies 1–16 years after original consultation is the best available. We consider that the bias against reply may also be higher in people who no longer have trouble, so the true overall outcome could be better than our survey suggests.

We believe careful pre-employment medical examination of prospective workers, good industrial hygiene, continuous health education of the worker and regular check-ups on the shop-floor for safety compliance are all-important.

In our opinion workers who contract dermatitis should be seen by a dermatologist as soon as possible, to receive the best available investigation and treatment. As soon as their condition allows it, they should return to and continue in their present job, unless they have contracted allergic contact sensitivity to materials in their working environment. In these latter cases a change of work is clearly indicated. There is of course a smaller group of patients who are never able to return to work. As the overwhelming majority of hand dermatitis is of the direct irritant type, change of work should rarely be necessary.

Industrial re-training of patients with hand dermatitis is time-consuming and expensive and the choices of occupations are limited for manual and especially for semi-skilled workers. In our opinion the results scarcely justify the efforts put into it and preventative work seems more rewarding.

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to have an effect on tumour microvasculature (Le Serve & Hellmann, 1972). The rationale for the use of razoxane in psoriasis is given elsewhere (Atherton *et al.*, 1980).

METHODS

Thirty-two patients were selected because of intractable cutaneous psoriasis. Sixteen of these patients had received methotrexate in the past, nine had not responded to this therapy and seven were intolerant of methotrexate. Three patients had severe arthropathic psoriasis.

In all patients baseline investigations included haemoglobin concentration, differential white cell count and platelet count. Biochemical screening of hepatic and renal function was performed along with the urinary creatinine clearance. Treatment was not instituted if the creatinine clearance was less than 75 ml/min. Atherton *et al.* (1980) also performed liver biopsies on all patients who had received methotrexate in the past.

Razoxane was given as 125 mg tablets, initially 750 mg usually on 2 or 3 consecutive days and increased at monthly intervals until clearance or dose-dependent side-effects occurred. Razoxane was stopped if the neutrophil count fell below $1{\cdot}2 \times 10^9$/l and recommenced at a lower dose. Patients were seen on a fortnightly basis while the dosage was incremental, and after a maintenance dose was achieved, the interval increased to up to 2 months. A clinical assessment of the psoriasis was made at each visit and haemoglobin concentration, white cell count and platelets were estimated. Biochemical screening of hepatic and renal function was performed on a 12-weekly basis.

In an attempt to minimize side-effects, Atherton had used various dosage schedules based on age, sex and weight (Atherton *et al.*, 1980). We looked to see which had proven most effective in the views of the physician and the patient.

The aim of therapy was to suppress the disease to a level acceptable to the patient, and adjuvant topical therapy with dithranol or other agents was used, if required.

RESULTS

In cutaneous psoriasis the initial response rate was 97% with clearance or regression to minimal disease in thirty-one out of thirty-two patients. The response time varied from 2 to 8 weeks before any improvement was seen. In 1982 we found that twenty-three (72%) patients remained on razoxane therapy at an average dose of 1032 mg per week (range 750–2250 mg). The average duration of therapy was 60·24 months (excluding one patient who stopped to conceive a family and then recommenced therapy). Of these twenty-three patients still receiving therapy, eight patients also required adjuvant topical therapy. All others were completely clear or had very minimal disease. Table 1 shows how the different subgroups of cutaneous psoriasis tolerated razoxane over the 6-year period.

The three patients with primary arthropathic psoriasis all continued on razoxane therapy with only one patient requiring regular anti-inflammatory drugs.

Nine patients (28%) with primarily cutaneous psoriasis discontinued therapy for the reasons shown in Table 2.

When we looked at dosage schedules we found that twenty-one patients were on consecutive day treatment, sixteen of these for 2 days per week and five on alternate day regimes. It is our impression that consecutive day therapy will provide the best balance between the clearing of psoriasis and development of unwanted side-effects.

TABLE 1. How the different clinical subgroups of psoriasis tolerated razoxane over the 6-year period

Subgroup	No. commenced on treatment	No. still on treatment
Nummular	11	7
Erythematous	15	12
Pustular		
Acropustulosis	2	1
Generalized pustular	1	1
Palmoplantar	3	2
Arthropathic	3	3

TABLE 2. Reasons for discontinuation of razoxane therapy

Patient	Reason for stopping treatment
1	Dose-dependent side-effects – diarrhoea
2	Dose-dependent side-effects – nausea and vomiting
3	Dose-dependent side-effects – neutropenia } on low doses insufficient to control psoriasis
4	Dose-dependent side-effects – neutropenia
5	Dose-dependent side-effects – neutropenia
6	Treatment withdrawn because of development of epitheliomata
7	Psoriasis quiescent with age
8	Stopped because of pregnancy (not recommenced because quiescent)
9	Stopped because of imminent surgery (not recommenced because quiescent)

We also noted several adverse effects with razoxane, some of which have not been reported before.

Side-effects

Bone marrow depression is seen in nearly all patients on razoxane. Neutropenia is most frequently seen and is often the dose-limiting factor. The nadir of the neutropenia occurs 3–10 days after the last dose. It is always reversible on stopping the razoxane, and the count rises within 14 days. Neutrophil counts were maintained above $1{\cdot}2 \times 10^9$/l by lowering the dose of razoxane as necessary. Before treatment the average neutrophil count was $6{\cdot}0 \times 10^9$/l and had fallen to $2{\cdot}67 \times 10^9$/l after treatment.

The average pretreatment haemoglobin was 14·9 g/dl and this fell to 13·4 g/dl. A macrocytosis was seen in nine patients and in all these cases the B_{12} and folic acid levels were estimated. An abnormally low folic acid was seen in two patients and five patients had levels at the lower end of the normal range.

Alopecia was seen to a mild degree in most patients. It was never severe and responded to dosage reduction.

Nausea was seen in 50% of patients but could be minimized by giving treatment over 2 days. In one case it was sufficiently severe to withdraw the razoxane.

Diarrhoea occurred in four patients and in one was sufficient to withdraw the razoxane. The others responded to a reduction in the dosage of razoxane.

Nasal discharge. A bloody discharge was seen transiently in twelve patients.

Leg ulceration was seen in three patients and has proved to be intractable in one patient.

Lethargy and headaches. Seven patients found this a disturbing side-effect. It was relieved by lowering the dose of razoxane.

Squamous epitheliomata. Two patients developed squamous epitheliomata on plaques of psoriasis (Horton, MacDonald & Wells, 1983). The two patients were extremely severely affected psoriatics who developed epitheliomata in non-sun exposed areas at the ages of 42 and 36 respectively. In one of these patients two squamous epitheliomata and six areas of *in situ* carcinoma were seen. The treatment was withdrawn in this case.

Five patients out of the whole group (14%) did not report any side-effects. In no case was hepatic dysfunction seen on biochemical screening.

DISCUSSION

Razoxane is a very effective drug in the treatment of all forms of psoriasis. Cutaneous psoriasis was completely cleared in many cases.

It was well tolerated over the follow-up period with only six (18%) of the patients stopping therapy because of adverse side-effects. Most of the side-effects are dose-dependent and the low maintenance dose required (average 1032 mg) tends to minimize these. It also appears that by giving the tablets over 2 consecutive days the adverse effects are reduced.

The most disturbing side-effect is of bone marrow suppression although this is reversible on stopping therapy. We advocate the use of special follow-up clinics for razoxane patients, with regular haematological checks as described above.

The previously unreported side-effects are those of the morphological changes in the red blood cells, leg ulceration and the development of squamous epitheliomata.

The macrocytosis seen in our patients did not appear to be related to a folic acid deficiency as it was not corrected after folic acid supplementation. Atherton *et al.* (1980) reported reversible bone marrow changes with razoxane therapy but did not report any morphological changes in the red blood cells themselves. It may be that these morphological changes are only seen after prolonged treatment and we hope to investigate this further.

The leg ulceration has been troublesome in one patient. Repeated biopsies of the lesion have been unhelpful and skin grafting did not aid healing.

The development of squamous epitheliomata on plaques of psoriasis is unusual and is discussed elsewhere (Horton *et al.*, 1983).

Although we were not able to perform serial liver biopsies in our patients, none developed hepatic dysfunction on biochemical screening. This includes the five patients who had hepatic abnormalities following methotrexate therapy and we feel that razoxane can be used safely in patients showing evidence of hepatic dysfunction.

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Epitheliomas in patients receiving razoxane therapy for psoriasis

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SUMMARY

We report two cases in which cutaneous squamous cell carcinomata developed in patients with psoriasis on razoxane therapy. The relevance of razoxane as well as past treatment for psoriasis in the aetiology of these tumours is discussed.

Razoxane [1,2-bis(3,5-dioxopiperazine-1-yl)propane] has been used in the treatment of severe psoriasis for 6 years, in some cases continuously. Early evidence suggested that the drug blocked the cell cycle at the early mitotic phase (M) or the late pre-mitotic phase (G_2) (Sharpe, Field & Hellmann, 1970). More recent work suggests that it may retard mitosis with failure of separation or rejoining of the daughter cells (Creighton, 1979). Inhibition of the cell cyle in this way would not be expected to produce mutagenicity, and when razoxane is subjected to the Ames test there is an absence of mutagenicity which correlates well with an absence of carcinogenicity (McCann *et al.*, 1975).

Despite this apparent absence of carcinogenic potential, we here describe two cases in which epitheliomata developed in patients on razoxane therapy.

CASE REPORTS

Case 1. A 42-year-old Caucasian male with a 29-year history of psoriasis had been treated for 25 years variously with courses of oral steroids, topical steroids, tar, dithranol, and 3 years of intermittent in-patient care. Following an episode of generalized pustular psoriasis, methotrexate produced a moderate therapeutic response. After 9 months, methotrexate therapy was discontinued because of liver toxicity, with consequent relapse of the psoriasis. PUVA therapy was instituted but was tolerated for 2 months only; he received a total dosage of 45 joules/cm^2, but this did not suppress his psoriasis. In 1979 the patient was commenced on razoxane therapy

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with good control of his disease on an average dose of 1500 mg per week. In August, 1982 he complained of ulcerated lesions of 7 weeks' duration on his arm and buttock. These proved to be squamous cell carcinomata. Six other suspicious lesions were biopsied and were shown to be areas of *in situ* carcinoma. A history of arsenic ingestion was denied and there was no evidence of arsenical keratoses or pigmentation.

Examination of haematoxylin and eosin (H & E) stained sections of the tumorous lesion from the buttock revealed considerable irregular proliferation of the eroded epidermis, with extension of individual invasive strands of epithelium deep into the dermis. Gross cytological atypia was present throughout the epidermis including many large, bizarre hyperchromatic cells, nuclear pleomorphism, numerous mitoses, dyskeratotic cells and loss of polarity. The features were characteristic of squamous cell carcinoma of the skin.

The lesion from the right arm comprised an extensive area of *in situ* carcinoma with marked parakeratosis, very numerous large hyperchromatic abnormal keratinocytes and considerable papillomatosis. Foci of normal epidermis were present at sites of hair follicles and sweat ducts. A single small area of invasive squamous carcinoma was present in the dermis.

Skin biopsy from the trunk showed three independent areas of carcinoma *in situ* separated by intervening areas of acanthotic but otherwise normal epidermis. Apart from foci of parakeratosis, cytological aberration was particularly present in the lower portion of the epidermis, giving rise to *in situ* epitheliomatous masses which protruded into the dermis.

Five other suspicious areas on the penis, chest, left arm, back, and left buttock were biopsied. These showed areas of *in situ* carcinoma with dysplastic change but no evidence of invasion.

Case 2. A 36-year-old Caucasian male with a 25-year history of gross psoriasis and a 12-year history of severe psoriatic arthropathy had been treated with courses of oral and topical steroids, tar and dithranol with little beneficial effect. He received methotrexate for an 18-month period, but this gradually became less effective in controlling his psoriasis. Methotrexate was replaced by PUVA, but after a 7-week trial period this was also found to be unsuitable. Razoxane was commenced in August 1977 and maintained at an average dose of 1125 mg per week. In November 1980 he developed an ulcerating nodule on a plaque of psoriasis on the right upper thigh.

Histopathological examination of the lesion showed gross epithelial proliferation comprising hyperparakeratosis, acanthosis and papillomatosis. Parakeratotic 'keratin pearls' were frequently present at the centre of the acanthotic masses. There was considerable loss of polarity and cellular pleomorphism of keratinocytes with large and hyperchromatic nuclei, numerous mitotic figures and dyskeratotic cells. Cohesion between epidermal cells was lost in places giving rise to foci of acantholysis. Individual strands and islands of this abnormal epithelium were seen extending deeply into the dermis. The appearances were diagnostic of cutaneous squamous cell carcinoma.

DISCUSSION

Epitheliomata in both cases arose in non-sun-exposed areas at a relatively young age. The two patients were extremely severely affected psoriatics and had received oral steroids, topical therapy with various compounds, including tar, dithranol and steroids as well as ultraviolet light, methotrexate and short-term PUVA, before starting razoxane.

There is no evidence that oral or topical steroids or dithranol are carcinogenic in man (Swanbeck & Hillstrom, 1971; Zetterburg & Swanbeck, 1971). It is well established that coal tar is a potential carcinogen (Berenblum, 1948) but the development of epitheliomata in treated patients is rare (Greither, Gisbertz & Ippen, 1967). Methotrexate administration over an 8-year period has shown no increase in the incidence of malignant disease (Bailin *et al.*, 1975). PUVA produced mutagenic aberration *in vitro* and *in vivo* (Swanbeck *et al.*, 1975; Swanbeck *et al.*, 1976), but both of our patients had received PUVA for less than a 2-month period, in low dosage.

The mechanism of action of razoxane is uncertain although it appears to cause blockage or slowing of the cell cycle (Sharpe *et al.*, 1970; Creighton, 1979). It also has an immunosuppressive action similar to cyclophosphamide (Dennert, Hatlen & Tucker, 1975).

In a previous report of a squamous cell carcinoma developing on a plaque of psoriasis, Harris felt that chronic immunosuppression was a factor favouring tumour emergence and growth (Harris, 1971). This theory of loss of immune surveillance may be important if previous therapy has produced a cumulative carcinogenic effect.

There are three reports which suggest a possible carcinogenic potential for razoxane. One report showed weak mutagenicity in mammalian cell culture lines incubated with razoxane (Witiak *et al.*, 1977). In another study large doses of razoxane administered intra-peritoneally to rats and mice resulted in an increased incidence of uterine adenocarcinomata and systemic lymphomata (DHEW Publication, NIH, 1978). Two cases of acute myelomonocytic leukaemia occurring in patients treated for a primary carcinoma with razoxane as a single agent, have also been reported (Joshi *et al.*, 1982).

There is no direct evidence that razoxane acts as a primary carcinogen *in vitro* or *in vivo*. However two of our seventy patients on this treatment have developed squamous cell carcinomata on covered areas of skin while relatively young. Our experience suggests that patients on razoxane for psoriasis must be carefully monitored for any evidence of malignant disease.

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Acne conglobata responding to buserelin, a gonadotrophin-releasing hormone analogue

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SUMMARY

A 65-year-old man who had had acne conglabata since early adult life was treated with buserelin for advanced prostatic cancer. Within 1 month of starting therapy, the inflammatory acne had completely resolved and this coincided with remission of the carcinoma. Gonadotrophin-releasing hormone analogues may be useful in the treatment of resistant acne.

The standard treatment of symptomatic advanced prostatic cancer is either orchidectomy or stilboestrol. Although both reduce symptoms in the short term, neither prolong survival and both have disadvantages (Blackard *et al.*, 1973; Lepor, Ross & Walsh, 1982). Buserelin, a long-acting analogue of gonadotrophin-releasing hormone, offers an alternative therapy for prostatic cancer without the cardiovascular risk of stilboestrol or the operative morbidity of orchidectomy. Long-acting analogues of gonadotrophin-releasing hormone act in a paradoxical way; after initially stimulating the release of pituitary gonadotrophins they subsequently reduce them (Bergquist *et al.*, 1979a; Bergquist, Nilius & Wide, 1979b). This is because the pituitary normally responds to the pulsatile release of endogenous gonadotrophin-releasing hormone from the hypothalamus (Belchetz *et al.*, 1978) but the long-acting analogues bind for longer than the endogenous hormone so that after initial stimulation the pituitary gonadotrophins become unresponsive (Swift & Crighton, 1978). Buserelin has been used successfully in the treatment of advanced prostatic carcinoma (Waxman *et al.*, 1983) and one such patient is reported whose lifelong acne resolved with buserelin.

CASE REPORT

A 65-year-old man presented with a 2-year history of malaise, weight loss and altered bowel habit. Since the age of 17 he had suffered from severe nodular and cystic acne affecting the back, chest and face. He had received several courses of long-term antibiotic therapy but the acne had remained active. On examination there were numerous open comedones, inflamed papules,

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pustules, cysts and scars on his back, chest and face. Histology of a prostatic needle biopsy showed a well-differentiated prostatic adenocarcinoma. The serum acid phosphatase was elevated at 98 KAU/100 ml (normal range < 2 KAU/100 ml). Computerized tomography of the abdomen showed massive prostatic enlargement and a diagnosis of T4 NO MO (UICC classification) carcinoma of the prostate was made. Treatment with buserelin was started at 200 μg five times daily given as an intranasal spray. One month later, the patient had improved subjectively with weight gain of 1 stone and relief of his malaise and constipation. Objective evidence of response was found both from computerized tomography of his pelvis which showed a decrease in prostatic size and from a reduction in serum acid phosphatase. Accompanying this response there was complete resolution of his acne and this has been maintained during 9 months of treatment. Temporary withdrawal of therapy by the patient caused an acute exacerbation of the acne.

DISCUSSION

Seborrhoea caused by androgenic stimulation of the sebaceous glands is an important factor in the pathogenesis of acne. The severity of the disease usually correlates with increased sebum excretion (Cunliffe & Shuster, 1969) but direct evidence that excess androgens are secreted in acne is sparse. In males, serum testosterone levels are no different from normal controls (Pochi *et al.*, 1965) but levels of the free active testosterone are not known since sex hormone binding globulin levels have not been measured. Abnormal end organ response to normal circulating androgens has been suggested by the finding of increased conversion of testosterone to

TABLE 1. Effects of buserelin therapy (200 μg five times daily)

	Pretreatment	After 1 month's therapy	Normal range
Sex hormone binding globulin	21 nmol/l	21 nmol/l	17–50 nmol/l
Testosterone	12 nmol/l	4·0 nmol/l	10–38 nmol/l
5α,3α-Androstenediol	0 76 nmol/l	0·45 nmol/l	< 0·8 nmol/l
Dehydroepiandrosterone sulphate	2150 nmol/l	1229 nmol/l	5120–8570 nmol/l
Dihydrotestosterone	1 28 nmol/l	0 78 nmol/l	2–6 nmol/l
Oestradiol	130 pmol/l	< 50 pmol/l	< 30–175 pmol/l
Progesterone	10 4 nmol/l	6 5 nmol/l	< 12 nmol/l
Prolactin	234 mU/l	164 mU/l	< 360 mU/l
LH-RH test			
Luteinizing hormone			
Time			
0 min	9·4 U/l	4·4 U/l	1 4–9·7 U/l
20 min	19 1 U/l	5 2 U/l	13 1–57·6 U/l
60 min	27 5 U/l	5 0 U/l	11–47 6 U/l
FSH-RH test			
Follicle stimulating hormone			
Time			
0 min	3 0 U/l	2 6 U/l	1–7 U/l
20 min	4·2 U/l	3 3 U/l	1–7 U/l
60 min	4 8 U/l	3 2 U/l	0 8–5·2 U/l

dihydrotestosterone in acne-bearing skin (Sansone & Reisner, 1971). Dihydrotestosterone is thought to be the active androgenic hormone in sebaceous glands.

The confirmation that androgens are major sebotrophic factors in the male comes from the reduction in sebum excretion rate with antiandrogens (supraphysiological doses of oestrogens or cyproterone acetate) or even orchidectomy (Hay & Hodgins, 1974; Burton, Laschet & Shuster, 1973; Pochi & Strauss, 1963). Each of these treatments have disadvantages and would not be recommended for long-term treatment.

The successful use of buserelin in this patient with acne non-responsive to long-term antibiotic therapy is of significance. The exact hormonal change that caused relief of the acne is uncertain since decreases in testosterone, dihydrotestosterone, $5\alpha,3\alpha$-androstenediol, dehydroepiandrosterone sulphate, oestradiol, progesterone, prolactin, luteinizing hormone and follicle stimulating hormone were observed (Table 1). The advantage of the treatment of acne with buserelin is that it is without the side-effects of oestrogens, cyproterone acetate and 13-*cis*-retinoic acid. In the dosage used in this patient mild facial flushing and decreased libido occurred, but a controlled trial of a lower dose of this agent in acne is now warranted.

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Comment

ULTRAVIOLET CARCINOGENESIS IN MICE AND MEN

Ultraviolet (UV) radiation is a major aetiological factor in the development of skin cancer. In man, skin neoplasia usually occur on light-exposed areas, and there is a clear correlation between geographical latitude and the incidence of skin malignancy. In experimental animals, skin cancers are readily induced by UV radiation. The wavelengths responsible are probably in the UV-B range (280–315 nm) although the action spectrum for this effect has not been fully elucidated. The dose–response relationships are not straightforward: thus the same total doses administered to mice at different rates do not result in the same frequency of tumour formation (Blum, 1959).

The malignant transformation is probably a result of damage to DNA mediated either directly by UV-B or as a consequence of error-prone repair mechanisms such as post-replication repair. The fate of transformed cells – whether they are recognized and eliminated or whether they flourish and progress – is largely determined by the competence of the immune system. This is reflected by the increased incidence of tumours of the skin and lymphoreticular system seen in immunosuppressed subjects such as renal homograft recipients. In addition, mice immuno-suppressed by drugs or methods which deplete T lymphocytes, develop UV-induced tumours sooner and in greater numbers than controls.

In studies of UV-carcinogenesis in mice, it was observed that UV-evoked cancers are usually strongly antigenic. Hence, if transplanted to normal recipients of the same strain (syngeneic), the tumours fail to grow and are rejected, whereas if transplanted to immunosuppressed or immunodeficient recipients, the tumours grow and eventually kill the host (Kripke, 1977).

These observations provoked the question of how UV-induced tumours escape immune destruction in the host in which they arise. It was shown that mice exposed to UV radiation lose their ability to reject transplanted tumours and become susceptible to tumour cells given either intradermally or intravenously (Kripke & Fisher, 1976). Thus UV radiation is not only directly tumorigenic but also causes a change in the immune system which renders the animal susceptible to the growth of such tumours.

Much work has been done to characterize the state of susceptibility. The wavelengths responsible lie between 270 and 315 nm, i.e. in the UV-B range. There is a surprisingly straightforward dose–response relationship; the proportion of animals becoming susceptible is proportional to the $\log_{10}$ UV dose (De Fabo & Kripke, 1980). Moreover, there is close reciprocity of effect between different dose rates so twelve separate exposures of 1 hour over a 4-week period have the same effect as one exposure of 12 hours. The susceptibility is thus acquired with much lower doses and long before carcinogenic levels are reached. The state of susceptibility has been found to persist for up to 6 months – a long time in the life of a mouse.

The state of susceptibility to transplanted tumours could be passively transferred: in experiments using parabiotic pairs it was found that a normal mouse joined to a UV-irradiated partner lost its ability to reject inoculated UV-induced tumour cells, whereas pairs of normal mice remained resistant (Fisher & Kripke, 1977). Attempts to transfer the susceptibility with serum or plasma from UV-irradiated donors failed. However, when the functions of lymphoid

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cells were examined by transfer to mice whose immune competence was destroyed by high dose X-irradiation, it was found that this susceptibility was indeed transferable. Thus, whereas lymphoid cells from normal donors reconstituted immune function in the X-irradiated mice so they were resistant to transplanted tumours, cells from UV-irradiated mice did not (Fisher & Kripke, 1978). These results might indicate that there is loss of some essential reactive cell type from UV-irradiated donors. However, when normal cells were mixed with cells from UV-irradiated donors and transferred to X-irradiated recipients, the recipients still proved susceptible to transplanted UV-tumours. This suggested an active suppressor mechanism and further evidence came from the observations by Spellman & Daynes (1977) that normal mice could be rendered susceptible by intravenous transfer of lymphoid cells from UV-irradiated donors. If the T cells were destroyed by treatment with an appropriate antibody and complement the suppressive capacity was lost, which indicates the cells responsible were suppressor T cells. There is now a substantial body of evidence confirming this.

Having thus established that the UV-induced susceptibility to transplanted tumours is due to suppressor T cells, various questions arise: Are they part of a generalized immunosuppression? What do they suppress? Are they important in the facilitation of growth of primary tumours that arise following UV irradiation? What is the mechanism of their formation?

To see whether the state of tumour susceptibility was a manifestation of general immunosuppression, a wide range of tests of the functional capacity of the immune system has been performed in susceptible, UV-irradiated mice (reviewed by Kripke, 1981). However, mice were normal in their ability to reject syngeneic non-UV tumours such as viral or chemically induced cancers, to reject skin and tumour grafts across major and minor histocompatibility differences, to form antibodies to a variety of antigens, to stimulate graft-versus-host (GVH) reactions, to respond *in vitro* to lymphocyte tests such as mitogen-induced proliferation and mixed lymphocyte reactions and to produce cytotoxic responses to various target cells. Although the immunocompetence of UV-irradiated mice is largely intact, certain defects were detected: they functioned poorly as recipients in local GVH reactions, and they failed to respond to the contact allergen dinitrochlorobenzene (DNCB). The significance of the last observation was not appreciated until more was known of the functions of Langerhans cells.

Evidence relating to the second question can be summarized as follows. Rejection of a transplanted tumour is accompanied by the appearance of cytotoxic T lymphocytes which are able to kill the tumour cells *in vitro*. If challenged with the same tumour again the mice mount a stronger secondary response and reject the tumour more rapidly. Once established, this specific immunity is not impaired by subsequent UV irradiation, (although this renders the animal susceptible to inoculations of different UV-induced tumour cells). Moreover, when transplanted to other mice bearing the specific tumour, such immune lymphocytes can suppress the growth of that tumour. *In vitro,* the cytotoxic reaction between immune lymphocytes and tumour cells is not inhibited by UV-induced suppressor cells. Thus the suppressor cells do not block the activity of established immune effector cells (i.e. the efferent component of the immune response). It appears rather that they act at some point in the induction or afferent phase. Hence, UV-irradiated mice are able to undergo minimal sensitization with tumour cells and can mount an initial cytotoxic response to small inocula of cells equal to that of unirradiated mice. However, their capacity to amplify the response with further stimulation is impaired and it is the generation of secondary, cytotoxic memory cells that appears to be inhibited (Thorn, 1978).

With regard to the third question, in experiments which separated the primary carcinogenic effects of UV from the possible contributions of immune mechanisms, Fisher & Kripke (1982) showed that the suppressor cells do indeed facilitate the growth of primary tumours: recipient mice were prepared by high dose X-irradiation and their lymphoid organs were repopulated with lymphoid cells either from normal or UV-irradiated donors. Four weeks later they were grafted with skin from syngeneic mice that received near-carcinogenic doses of UV-B. The subsequent development of cancers in the irradiated skin was much increased in the mice reconstituted with suppressor-rich lymphoid cells from UV-irradiated donors. In a second experiment, during the course of UV irradiation mice were injected at 3-weekly intervals with lymphoid cells from either normal or UV-irradiated donors, so suppressor cells were present in increased numbers from earlier on. The tumours developed in only half the time in the mice injected with suppressor cells.

The mechanisms by which UV-B evokes the formation of suppressor cells centre upon epidermal Langerhans cells (LCs). These are members of the immune system, which present antigens to the lymphoid system in the induction of T-cell mediated responses such as contact hypersensitivity. They are peculiarly sensitive to UV-B and after even quite small doses of irradiation they can no longer be demonstrated by the usual membrane-associated markers such as ATPase or presence of Ia antigens (Toews, Bergstresser & Streilein, 1980; Aberer *et al.*, 1981). In addition, their functional capacity is dramatically altered after exposure to UV-B so that they lose the ability to stimulate active T-cell mediated hypersensitivity to test antigens and, instead, elicit specific unresponsiveness or tolerance. Thus, if contact sensitizers such as DNFB are applied to mouse skin depleted of LCs by exposure to UV-B, the mice become tolerant to DNFB so that subsequent attempts to induce sensitivity fail and they remain unresponsive (Toews *et al.*, 1980). This form of tolerance has been shown to be due to suppressor T cells. The nature of the alteration induced in LCs by UV-B remains to be determined.

The hypothesis that links these events and the induction of cancers by UV-B invokes three separate steps. First, UV-B in relatively small doses induces the formation of new antigenic determinants on epidermal cells which either resemble or may be the common or shared antigens of UV-tumours. Secondly, these new antigens are presented to the immune system by LCs whose function has also been impaired by the UV-radiation. This results in the generation of suppressor T cells which will inhibit the future formation of memory T cells directed at those tumour-related antigens. In the third step, higher doses of UV-B induce malignant transformation of epidermal cells and hence initiate tumour growth. Since the normally protective mechanisms are blocked by the state of susceptibility, the tumour(s) grows.

The events described above have been elucidated in mice exposed to high dosage UV radiation – repeated exposure to 540 mJ/cm^2, about ten times the maximum that could be tolerated by man (Diffey, Larko & Swanbeck, 1982). However, it is not unreasonable to suppose that similar mechanisms may operate in man and there is evidence which supports one part of the story: human LCs are damaged by low doses of UV-B (Aberer *et al.*, 1981) and UV-treated subjects sensitize poorly with DNCB (Moss, Friedmann & Shuster, 1981). It is not known whether the poor sensitization corresponds to the tolerance observed in mice. Unfortunately, the methods usually used to evaluate immune competence are unlikely to yield the necessary evidence since the defect is so specific and even in mice was not detected by a host of tests of immune integrity.

If we adopt a working hypothesis that similar mechanisms exist in mice and men, then it is likely that therapeutic or holiday exposure to UV is cocking the trigger of susceptibility in many people. It is especially worrying that this effect may not be preventable: sunscreens that can protect against sunburn cannot prevent the damage to LCs or the development of susceptibility

(Lynch, Gurish & Daynes, 1981). This may be because the relatively small doses of UV-B which can damage LCs are not filtered out by the sunscreens.

On the other hand, it must be remembered that the doses used to produce these changes in mice are large compared with therapeutic dosages. Therefore, until the mechanisms of the UV-induced disturbances of immune responsiveness in man are elucidated an alternative hypothesis must be retained which allows for different mechanisms in mice and men.

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PAPERS

Sequential studies of the cutaneous infiltrate in mycosis fungoides

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Sequential studies using monoclonal antibodies to membrane markers of T-cell subsets, Langerhans cells/indeterminate cells and HLA-Dr antigens were carried out on a total of fifty skin biopsies from fourteen patients with histologically proven mycosis fungoides (MF) and four with already suspected but histologically not yet proven MF.

Frozen sections were treated with monoclonal antibodies to T-cell subsets, dendritic cells and HLA-Dr antigens using the peroxidase technique. A double staining technique was also used in some specimens.

The immunopathological results closely parallel both the clinical picture and routine histological findings. In seventeen patients on PUVA therapy, biopsies at least 48 hours post-treatment showed a reduction in the number of Langerhans cells/indeterminate cells in the epidermis compared with the pre-PUVA biopsy or our normal range, along with a decrease in the number of OKT4+ (helper) T lymphocytes and especially OKT8+ (suppressor/cytotoxic) lymphocytes. Staining of epidermal keratinocyte cell membranes with anti HLA-Dr of varying degree was seen in most biopsies, persisting in some after clinical and histological improvement was seen.

Radiotherapy treatment received by two patients led to a decrease in both helper and suppressor lymphocytes in the dermis but only a slight reduction in the dendritic cell population in the epidermis.

In four biopsies histologically only suspected of being MF the immunopathological results suggested early MF before histopathological findings were considered diagnostic. In each case the diagnosis was proven by clinical developments and subsequent biopsies.

This study is the first to monitor the effects of PUVA and radiotherapy on all cell types considered to be involved in mycosis fungoides.

Cutaneous T-cell lymphoma—an *in situ* study using monoclonal antibodies

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A series of monoclonal antibodies against normal and malignant T cells was used to examine *in situ* the cutaneous infiltrate of cutaneous T-cell lymphoma (CTCL). Ten patients with CTCL from stages I-IV,

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and a control population of ten patients with a variety of benign lymphocytic infiltrates were examined using an indirect immunoperoxidase reaction.

In all sections, a pleomorphic infiltrate of cells was present with the predominant cell being the T cell (OKT1+) and the majority OKT4+ (helper T). Up to 20% of the T cells were reactive with OKT8, (suppressor T) and a smaller population of cells reactive with OKT6 (Langerhans cells) was present.

In the benign lymphocytic infiltrates, a larger proportion of OKT8+ T cells was present (up to 50%).

BE1 and BE2 (tumour-associated antigens) reactive cells were present in the dermal and epidermal infiltrates of all patients with CTCL. The number of reactive cells varied from $<10\%$ to 90%. No mononuclear cells reactive with BE1 or BE2 were identified in any of the benign dermatoses.

This study confirms the limitations of anti-T cell monoclonal antibodies in the tissue diagnosis of CTCL, but demonstrates the value of BE1 and BE2 in the early diagnosis of CTCL.

T lymphocytes in experimental murine dermatophytosis: Adoptive cell transfer and resistance to the disease

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A mouse model was established for investigating the immunological basis of cutaneous dermatophytosis due to *Trichophyton quinckeanum* (Calderon & Hay, *Br. J. Derm.* 1983, **108**, 221). The *in vivo* adoptive cell transfer system which allows manipulation of cells prior to their transference into syngeneic irradiated recipients was employed. The intensity and duration of dermatophyte lesions in Balb/c mice were significantly increased after being subjected to irradiation (550 R). Lymph node and spleen cells from pre-infected donors conferred significant protection to irradiated recipients when injected intravenously 24 h before infection of the skin. The capacity of these cells to transfer adoptive immunity was lost after treatment with monoclonal anti-Thy-1 antibody and complement, demonstrating that resistance to infection can be immunologically mediated by T-cell co-operation.

Since the prime concern in human dermatophytosis is the chronicity of the infection, we therefore studied the cellular activity of chronic infected mice using the cell transfer system. Cells from these animals which were initially supposed to be immunologically compromised, did however confer adoptive immunity. Further studies on the presence of 'blocking factors' in the original chronically infected host which may interfere with the expression of cellular immunity are being undertaken.

Quantification *in situ* of T-lymphocyte subsets in atopic eczema

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Patients with atopic eczema (AE) are known to have functional and numerical deficiencies of circulating T-suppressor cells. We have used a range of monoclonal antibodies in an indirect immunoperoxidase evaluation of frozen skin taken from ten AE patients. In particular we have quantified T-lymphocyte subsets and Langerhans cell populations in the dermal infiltrate.

Five-micron sections were air-dried, rehydrated and incubated with 1:10 dilutions of monoclonal antibodies UCHT1 (pan T cell), Leu 3a (helper), OKT6 (Langerhans cell), Leu 7 (natural killer) and OKT8 (suppressor) for 1 hour at room temperature. Secondary incubation was performed using human absorbed HRP-conjugated sheep anti-mouse Ig antibody and the peroxidase activity was revealed by 3′,3-diaminobenzidine and hydrogen peroxide.

Ten high power fields of duplicate sections were examined by two observers independently using a Zeiss microscope fitted with an eye piece graticule. Whereas helper T cells comprised 86% of the total T lymphocyte count, suppressor T cells amounted to only 26%, with a helper/suppressor ratio of 3·29. Of particular interest was the high number of Langerhans cells (11% of the total T-cell population). Natural killer cells were not demonstrated.

The high helper/suppressor ratio reflects those changes noted in the peripheral blood of AE subjects and may be related to a suppressor T cell deficiency. Lack of suppression of immune responses to common antigenic stimuli may be responsible for the early inflammatory changes noted in AE. Furthermore the high concentration of Langerhans cells may augment this response by increased antigen presentation.

Evidence of decreased natural killer (NK) cell activity in patients with atopic dermatitis—a sequential study

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Natural killer (NK) cell activity is thought to be restricted to a population of large granular lymphocytes. *In vitro*, these cells have the ability to lyse spontaneously certain tumour cells and also some virally infected cells. Interferon has been reported to augment their activity.

Patients with atopic dermatitis have a number of immunological defects, both humoral and cell mediated. It is well recognized that some patients with atopic dermatitis are unable to handle certain viruses normally. Herpes simplex infection, and vaccination against smallpox can result in Kaposi's varicelliform eruption, a severe and potentially fatal condition.

We therefore tested the hypothesis that NK cell activity may be deficient in the peripheral blood in patients with atopic dermatitis. NK cell function was assessed by a chromium release assay. The patients' cells (effector cells) were tested against a chromium-labelled K562 lymphoblastoid cell line (target cells). Results in twenty atopic patients demonstrated a significant reduction in NK cell function as compared with normal age-matched controls ($P < 0{\cdot}0032$ by Mann-Whitney test). No relation between NK cell activity and IgE, log IgE and clinical scores could be demonstrated.

Eight patients have been studied sequentially over 6 months to confirm these findings.

Sequential studies in bullous pemphigoid

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Bullous pemphigoid is an organ-specific auto-immune disease which is usually self-limiting. We have performed sequential studies on fifty-four patients with pemphigoid and found that they tend to fall into two distinct groups: those with high basement membrane zone (BMZ) antibody titres (> 1:160) and those with low antibody titres. Over a period of 2 years those with high titre antibodies remained high though they diminished slowly: in those with low titres, the BMZ antibodies usually became unmeasurable after 4 months. There was no direct correlation with the disease extent or activity in either group. Those patients with high titres of BMZ antibodies were those who had high total serum IgE concentrations and there was a highly significant correlation between the two parameters ($P < 0{\cdot}001$).

Forty-five per cent of patients had an intitial peripheral blood eosinophilia, and 73% of those with an eosinophilia had raised serum IgE concentrations. There was also a significant correlation between serum IgE and serum IgG_4, though the specificities of these antibodies remain to be elucidated. Serum IgE was raised in 67% of patients who presented with pruritus compared with only 31% who did not ($P < 0{\cdot}02$).

The pathogenesis of pemphigoid remains to be determined: being a self-limiting disease it is likely that a single factor such as a virus infection may be the triggering factor. Further studies of IgE and IgG_4 in pemphigoid may help to elucidate this.

Circulating gliadin–antigliadin immune complexes in dermatitis herpetiformis (DH) and coeliac disease (CD)

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Many immune complex detection methods have been used in CD and DH, but each has suffered from the drawback that components of the complex are not identified. In this study sucrose density centrifugation was used to fractionate sera from nineteen adults with DH [eight on a gluten-free diet (GFD) and eleven on a normal diet] and six adults with CD (three on a GFD and three on a normal diet), according to molecular weight, so as to separate heavy or complexed food antibody, from light uncomplexed food antibody. Each fraction was tested for the presence of IgA, IgG and IgM and for anti-gliadin antibody of each class. In some cases fractions were also screened for candida antibodies. Heavy gliadin antibody activity was found in the majority of patients on a normal diet, relating to the state of the gut, but rarely in those on a strict GFD. The heavy antibody was only of IgG class and never IgA or IgM. Candida antibodies, however, were of all classes and sedimented exclusively in the low molecular weight zone. These results suggest that a leaking gut mucosa allows food antigen entry and the subsequent formation of immune complexes. They do not support the view that IgA anti-gliadin complexes account for the skin depositis of IgA in patients with DH.

Induction of tolerance to urushiol by epicutaneous application of this hapten on DNFB-treated skin.

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In a previous paper we pointed out that epicutaneous application of a sensitizing dose of DNFB on guinea-pigs decreases the density of ATPase-positive Langerhans cells (LC). This density reaches its lowest level at the 24th hour. In fact most of the LC lost only their enzymatic marker (ATPase) since most of them remained morphogically unaltered as determined by electron microscopy.

Immune tolerance to a second hapten (urushiol) is induced by application of the latter on the DNFB-treated skin area.

Seven DNFB-treated guinea-pigs were sensitized at the 24th hour (day 1) with urushiol (on the DNFB-treated skin area). Challenge tests with urushiol were made on day 15. On day 18, the guinea-pigs were subjected again to an immunizing dose of urushiol on a normal skin area. Challenge tests were carried out on day 32. Seven animals were sensitized to urushiol (control group). Results are summarized in the following table.

	Sensitization to urushiol	Intensity of the challenge test to urushiol ++	+	0
Control group	On normal skin	7/7	0/7	0/7
Experimental	DNFB-treated[a]	1/7	4/7	2/7
group	On normal skin[b]	2/7	3/7	2/7

[a] Challenge on day 15.

[b] Challenge on day 32.

This study shows that DNFB-treated skin is unable to promote normal sensitization to urushiol Epicutaneous application of urushiol results in the development of unresponsiveness or lowers the intensity of sensitization.

Early cellular events in contact sensitivity induced by dinitrochlorobenzene

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We have investigated the entry of dinitrochlorobenzene (DNCB) into the skin of previously sensitized human subjects, and studied subsequent cellular events.

Biopsies were taken from standard challenge sites at various time intervals after application of DNCB. With an antibody to DNCB and monoclonal antibodies to epidermal Langerhans cells, Ia antigens and T-cell subsets, indirect immunofluorescence and immunoperoxidase techniques were used to identify the sensitizer and cell types in 5-μm sections. Routine electron microscopy and immunoelectron microscopy were also performed.

DNCB penetrated the skin rapidly, and by 2 hours reached the dermo-epidermal junction, where it was retained. At 6 hours, numerous DNCB-positive cells, some dendritic, were seen in the dermis. From 3 hours, Langerhans cells assumed a more basal position in the epidermis, and at 6 hours, many large OKT6 and Ia-positive cells, many dendritic, were found at all dermal levels. The epidermal dendritic pattern of Langerhans cells was disrupted, and cell numbers decreased at 12 hours, with some recovery by 24 hours, when apposition to T cells was seen within the epidermis.

We have shown, using a specific anti-DNCB antibody, that the sensitizer enters the epidermis rapidly and penetrates further within the first 24 hours in a cell-bound form, corresponding to the appearance in the dermis of cells with Langerhans cell characteristics.

The incidence of autoimmune-related disease in men with lichen sclerosus et atrophicus

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Coat colour changes and tyrosinase activity in the Agouti mouse

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Agouti mice acquire their first coat of hair, which is golden yellow in colour, at 10–15 days of age. At puberty (32–35 days) they grow a darkly pigmented coat which in later life is gradually replaced by a golden yellow coat. The reason for these changes in coat colour is unresolved. In this study we have measured changes in tyrosinase activity in the skin of Agouti mice at different stages of coat colour using a modification of the method described by Pomerantz (*J. Biol. Chem.* 1966, **241**, 161).

No tyrosinase activity was found in adult mice (70 days of age) with a golden yellow coat but there was an elevation in tyrosinase levels in the pubertal mice (35 days of age) that were growing a dark coat. A similar level of activity was found in 5- to 15-day-old animals that were growing their first golden yellow coat. This suggests that changes in tyrosinase are not associated with coat colour but may be related to hair growth.

We therefore examined the effect of plucking, known to initiate new hair growth, on tyrosinase activity. Hair was plucked from both adult mice with a golden yellow coat and from pubertal mice with a dark coat. After 16 days both groups had regrown hair which was similar in colour to their pre-existing hair. Increases in tyrosinase activity were found in both groups of mice, starting at around 4–6 days after plucking, although the peak value in the adult mice was not as great as that in the pubertal mice.

Tyrosinase activity is therefore not necessarily associated with the appearance of dark hair but may be related to the growth of new hair. Increases in tyrosinase activity are not confined to the appearance of dark hair and this suggests that other factors are involved in the control of coat colour in the Agouti mouse.

Plasma 5-S-cysteinyldopa as an index of melanogenesis

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A high proportion of melanoma cells retain the property of melanogenesis, even after secondary spread. 5-S-cysteinyldopa (5-SCD) an intermediate in the synthesis of melanin, has been detected in urine and plasma and increased urinary excretion has been found in patients with secondary melanoma (Agrup *et al.*, *Acta dermato-vener.* 1977, **57**, 113). The aims of this study were: (1) to measure 5-SCD in plasma and examine whether changes in skin melanogenesis are reflected in 5-SCD concentrations and (2) to establish reference values for plasma 5-SCD.

5-SCD was measured by high pressure liquid chromatography with electrochemical detection. The precision of the method is 15% (c.v.) and it is sufficiently sensitive to detect 5-SCD in plasma from normal individuals. Patients having ultraviolet radiation therapy for psoriasis had normal 5-SCD concentrations and there was a rapid rise in 5-SCD within 10 to 20 days, the maximum being 200–500% of pretreatment values. 5-SCD fell thereafter to levels 25–50% above pretreatment. In healthy subjects the fasting 5-SCD concentration was 4·6–6·0 nmol/l. There was no significant diurnal variation; meals had no effect. An extended study of 5-SCD concentrations so far shows little change (winter and spring months). 5-SCD concentrations in plasma from healthy volunteers of Asian and African origin were not significantly different from those in Caucasians.

These studies show that plasma 5-SCD reflects the degree of skin melanogenesis. Diurnal and seasonal variations are, so far, insignificant. The value of these measurements in patients with primary and secondary melanoma is being studied.

Calcium, cyclic AMP and MSH-stimulated melanosome dispersion

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MSH stimulation of melanosome dispersion is mediated via cyclic AMP (cAMP). Calcium is also required, but its mode of action is unknown. It has been suggested that Ca^{2+} is necessary for membrane transduction of the MSH-signal but our evidence suggests that Ca^{2+} has several points of action in regulating melanosome dispersion in the lizard, *Anolis carolinensis*.

We found a parallel shift in α-MSH dose–response curves (50 pM–70 nM) by changing the Ca^{2+} concentration (10^{-5}–10^{-1} M), indicating the Ca^{2+} acts at the same point as MSH. Using an analogue of α-MSH, Nle^4-D-Phe^7-α-MSH, which binds irreversibly to the MSH-receptor, we have shown that Ca^{2+} is required for initial receptor–peptide binding. Once bound, the darkening effect of the peptide could be abolished by removal (+ 10^{-4} M EGTA) and restored by addition of Ca^{2+} (10^{-3} M), suggesting that Ca^{2+} is also required for mediating the effect of the peptide after binding. The Ca^{2+}ionophore A23187 ($>1{\cdot}6\times10^{-5}$ M) showed that a Ca^{2+}-influx could stimulate melanosome dispersion. Ca^{2+}-influx inhibitors verapamil (5×10^{-5} M) and nifedipine (3×10^{-5} M) also reduced the potency of α-MSH. These results suggested that Ca^{2+} was acting as either an additional second messenger to cAMP or in transducing the MSH signal to adenylate cyclase (AC). Forskolin ($>3\times10^{-8}$ M), a potent activator of the catalytic subunit of AC, stimulated melanosome dispersion. Although active in the absence of extracellular Ca^{2+}, its potency was reduced by the calmodulin antagonist W7 (100 μM), suggesting that there was a Ca^{2+}-calmodulin requirement distal to AC. W7 also reduced the action of cAMP (Tris salt), suggesting that Ca^{2+} can act at a point distal to cAMP production.

While Ca^{2+} may be necessary for MSH-signal transduction, our evidence suggests that it is also required for: (1) MSH receptor binding, (2) activation of adenylate cyclase and (3) the action of cAMP.

Tolerance to H_1 receptor antagonists

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Patients were shown to develop a dermal tolerance to the antihistaminic effects of the early histamine H_1 receptor antagonists which developed 7 to 14 days after treatment was started. This trial has reassessed this phenomenon using a new H_1 receptor antagonist, clemastine, and has investigated the possible role that the H_2 receptor might play in the development of H_1 receptor antagonist tolerance.

A single oral dose of cimetidine 400 mg and a matching placebo was administered in a double-blind manner on separate days to nine healthy male volunteers. The mean area of the weal and flare response to four 1-μg intradermal injections of histamine base was measured. Clemastine (Tavegil®) 1 mg b.d. was then commenced; after 3 days the histamine weal and flare responses were reassessed. After 21 days of clemastine therapy the effects of cimetidine 400 mg and the matching placebo upon the weal and flare responses were measured in a double-blind fashion on separate occasions.

The area of the weal response after 3 days on clemastine therapy was significantly smaller than after 21 days ($P<0{\cdot}01$), while the flare response was decreased in area to a similar extent on both occasions. There was no significant difference between the area of the weal response measured after a single dose of cimetidine 400 mg, given alone, and that recorded when cimetidine was added to clemastine therapy.

The subjects' histamine-induced weal response was shown to develop a tolerance to the H_1 receptor antagonist clemastine after 21 days of therapy, despite sufficient concentrations of clemastine being present in the skin to maintain the suppression of the flare response. H_1 receptor antagonist tolerance did not appear to be overcome by the addition of the H_2 receptor antagonist cimetidine.

The effect of intradermal infusions of 12-hydroxy eicosatetraenoic acid (12-HETE) in normal human skin

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12-HETE, the 12-lipoxygenase product of arachidonic acid, is chemokinetic and chemotactic for human neutrophils *in vitro*, but its *in vivo* effects have not been described. 12-HETE is present in lipid extracts of scale and chamber fluid from involved and uninvolved skin of patients with psoriasis and may well be relevant to the pathogenesis of the psoriatic neutrophil infiltrate. We have investigated the *in vivo* effect of 12-HETE by intradermal infusion into the normal skin of six healthy volunteers. Two subjects received biologically synthesized and four subjects chemically synthesized 12-HETE using infusion of phosphate-buffered saline (PBS) pH 7·4 and the chemokinetically inactive 9-hydroxy-octadecadienoic acid (9-HODD) as controls.

12-HETE (0·39 mM) or an equimolar concentration of 9-HODD or PBS were infused intradermally using a syringe driver at a rate of 40 μl/h for 8 h, at which time biopsies were taken. The pattern of the intradermal infiltrate was assessed histologically and the infiltrate analysed quantitatively.

Infusion of 12-HETE produced a marked neutrophil and mononuclear cell infiltrate around vessels and in tissue spaces, whereas 9-HODD and PBS produced only a very slight increase in inflammatory cells.

Quantitative analysis revealed the presence of 20·36 ± 1·71 (mean ± s.e.m.) neutrophils and 5·92 ± 0·53 mononuclear cells per high power field (HPF) for 12-HETE and 0·9 ± 0·26 and 0·2 ± 0·0133 neutrophils and 0·4 ± 0·093 and 0·5 ± 0·27 mononuclear cells per HPF for PBS and 9-HODD infusions respectively. The quantitative difference between control and 12-HETE infusions was significant ($P = < 0·01$).

This study demonstrates biologically and synthetically produced 12-HETE is capable of eliciting a leukocyte infiltrate *in vivo* in man and provides further evidence that 12-HETE may be an important inflammatory mediator in psoriasis.

Acetylator phenotype in discoid lupus erythematosus

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Drug acetylation is bimodally distributed and slow rates of acetylation are associated with development of both idiopathic and drug-induced systemic lupus erythematosus (SLE). Since some patients with discoid LE (DLE) develop SLE we have examined the relationship between DLE and acetylator phenotype, which was determined in forty-three patients with DLE diagnosed clinically and histologically, and in fifty-one healthy subjects. All subjects had normal renal function as shown by blood urea and creatinine concentrations and none were taking a drug metabolized by acetylation at the time of the study. The ratio of acetylated to total drug (metabolic ratio, MR) was measured in urine collected from the 5th to 6th hour following 1 g oral sulphadimidine. The results show a clear bimodal distribution of acetylator phenotype in both DLE patient and normal populations, no subject having an MR between 0·70 and 0·80. Of the forty-three patients with DLE 58% (25) had an MR of less than 0·70 (slow acetylator) compared with 57% (29) of the normal subjects, and 42% (18) had a ratio greater than 0·80 (fast acetylator) compared with 43% (22) of the controls. However of the twenty-one DLE patients who had some evidence of systemic changes but not amounting to SLE (ANF and/or Raynaud's and/or leucopenia and/or arthritis) fourteen (67%) were slow acetylators (NS). Median MR in slow acetylators with SLE was 0·53, in slow acetylators with DLE it was 0·60, and was 0·57 for controls; median number of lesions was 8·5 in slow acetylators with SLE and was 3·0 in fast acetylators with SLE. These differences are not significant probably because of small numbers but they suggest a causal relationship between disease expression and speed of acetylation in LE.

Interferon in the treatment of persistent viral warts

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The aim of this work was to establish in an uncontrolled pilot study the potential value of interferon therapy of viral warts.

Seven patients with common and/or plantar viral warts present for at least 1 year (mean duration 5·5 years) and resistant to previous vigorous therapy were studied. Ethical committee approval and written informed consent were obtained prior to entry of patients. Doses of interferon ranging between 0·5 and 1 mega units were injected intra-lesionally into the most prominent or troublesome wart twice weekly for 6 weeks and the patients were fully reviewed 2 weeks and 6 weeks after cessation of treatment. Patients were assessed at each visit to the clinic and a detailed record was kept of clinical progress and adverse reactions. Tracings were made and photographs taken at regular intervals.

During the assessment period the warts of four patients receiving intra-lesional injections were completely cleared. The wart of one further patient cleared and then partially recurred and of the other two patients, one experienced partial clearance and the other's wart was unchanged. In the three cases who had multiple warts at sites distant to the intra-lesional injection all experienced either completed or partial clearance of these lesions. Although complete clearance tended to occur only after several weeks of treatment, changes in the warts indicating progress were often observed after only a few injections. All patients found the intra-lesional injections to be painful but no local necrosis occurred. Most patients experienced some 'flu-like symptoms but these problems were usually confined to the day of the injection and were usually relieved by taking paracetamol tablets on the day of the injection.

Only the most difficult cases were entered into this study and therefore the results are very encouraging and indicate that further work to evaluate more fully the role of interferon in the treatment of viral warts is warranted. We have already commenced a pilot study involving twice-weekly intramuscular injections of interferon and should this provide positive results a fully controlled study will be instituted.

Comparison of red cell deformability and electrophoretic motility in systemic sclerosis and Raynaud's disease

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We have shown that the deformability and electrophoretic motility of red blood cells (RBC) from patients with progressive systemic sclerosis (PSS) and Raynaud's phenomenon are reduced compared with normal controls (Kovacs *et al., Clinical Sci.* 1983, in press). We therefore were interested to examine the red cell behaviour of patients with Raynaud's disease (RD) and compare these findings with those from patients with PSS.

Deformability of RBC was measured by a filtration technique through sieves of pore diameter 3 μm with negative pressure (expressed in ml/min), and 5 μm diameter under gravity (expressed as relative resistance). RBC motility was measured by moving boundary electrophoresis (Streichman *et al., Br. J. Haematol.* 1981, **48**, 273).

The filterability of RBC (through a 3-μm filter) from twenty-four patients with PSS was reduced, $0{\cdot}224 \pm 0{\cdot}0168$ ml/min compared with twenty-three patients with RD, $0{\cdot}466 \pm 0{\cdot}0247$ ml/min ($P < 0{\cdot}001$). With a 5-μm filter and gravity alone, the filterability was still reduced in sixteen patients with PSS, $0{\cdot}420 \pm 0{\cdot}029$ compared with seventeen RD patients, $0{\cdot}509 \pm 0{\cdot}022$ ($P < 0{\cdot}05$). These measurements were also performed at different temperatures.

The electrophoretic motility of the ascending and descending boundaries were $0{\cdot}542 \pm 0{\cdot}014$ mm/min and $0{\cdot}605 \pm 0{\cdot}014$ mm/min in twenty-four patients with PSS and $0{\cdot}643 \pm 0{\cdot}023$ mm/min and $0{\cdot}725 \pm 0{\cdot}023$ mm/min in fifteen patients with RD. Differences between corresponding boundaries were significant at $P < 0{\cdot}001$.

This study has shown that RBC from patients with PSS are less deformable than, and have a reduced electrophoretic motility compared with, RBC from patients with RD. These additional investigations may be useful in the differentiation of patients with RD and PSS.

An investigation into the sebum excretion rate above and below the lesion in paraplegic patients

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We have observed that the incidence of acne on the back and buttocks appeared greater in paraplegic patients than in normal subjects. The sebum excretion rate (SER) was therefore investigated in seventeen male and four female subjects aged from 16 to 64 years, and in a similar number of age-matched controls.

Measurements were made on both the forehead and the anterior thigh using a slight modification of the quantitative gravimetric method (Strauss & Pochi, *Journal of Investigative Dermatology*, 1961, **36**, 293) using vertical flaps to hold the absorbent papers in position, plus a crepe bandage encircling the head.

On the forehead the range was 0·70–1 68 (mean 1·11 mg/10 $cm^2/3h$) for paraplegic females compared with 0·61–1·90 (mean 1.22 mg) for controls. Male paraplegics produced 0·61–3·6 (mean 1·69 mg) compared with 1·15–2·57 (mean 1·68 mg) for controls. A two-tailed t-test revealed no significant difference ($t=0{\cdot}64$) between paraplegics and controls. On the thigh the range was 0·40–0·73 (mean 0·56 mg) for paraplegic females compared with 0·20–0·34 (mean 0·28 mg) for controls. Male paraplegics produced 0·30–1·13 (mean 0·57 mg) compared with 0 14–0·47 (mean 0·28 mg) for controls. A two-tailed t-test revealed a highly significant difference ($t=6{\cdot}27$) between paraplegics and controls.

We have therefore shown a previously undescribed increased SER below the neurological lesion in paraplegic patients, accounting at least in part, for their increased incidence of acne.

Differences in the inflammatory response in male and female patients with acne vulgaris

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In acne vulgaris one sees the progression of non-inflamed to inflamed lesions but the initiators and mediators responsible for this progression remain uncertain. However, it is thought highly likely that *Propionibacterium acnes* and/or its products are prime candidates for the initiator role. A major factor influencing this inflammatory response could be the competence of the host's defence system to deal with this assault. To investigate this we have used various antisera (including the OKT-monoclonal antibodies) to estimate the subpopulations of peripheral blood leukocytes, C3 and other serum protein levels in normal subjects and acne patients.

This study showed that the earliest significant events in acne were the mobilization of neutrophils and increased levels of complement C3. Although the pattern of responses of male and female acne populations were similar they were out of phase with each other. In females, the rise in levels of these mediators occurred in the mild acne group, whereas in males such a response was only evident in the moderate grades.

The increased severity of the acne condition correlated with increased numbers of helper T cells, but there was no change in numbers of suppressor T cells. Again the responses in females were more rapid with significant helper T cell activation in moderate acne, whereas in males this was only evident in severe acne. Also significantly increased B-cell numbers (humoral responses) were only found in males with severe acne.

This evidence indicates that females produce their inflammatory responses much earlier than males, controlling their acne more readily and not allowing it to progress to the more chronic condition seen in males.

A comparison of the effect of combinations of oestrogen and cyproterone acetate and oestrogen and norethisterone on the androgens of women with acne

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Fifty-one female patients with acne (mean age 23 years) of whom 43% were taking an oral contraceptive pill prior to the study were stratified and randomized into one of three therapeutic groups, all of whom received ethinyloestradiol 50 μg (days 5–25). In addition group 1 received cyproterone acetate 50 mg (days 5–14), group 2 cyproterone acetate 2 mg (days 5–14) and group 3 norethisterone 1 mg (days 5–25 of the menstrual cycle). The total and unbound serum testosterone and dihydrotestosterone (DHT) were estimated mid-cycle prior to treatment and in the sixth cycle of therapy and correlated with clinical improvement and fall in sebum excretion rate.

The results prior to treatment showed a wide scatter of values (all expressed in pg/ml) both within and above the normal range (mean ± s.e.m.). The free testosterone was 4·18 ± 0·382 (normal range < 5·0) and free DHT 1·85 ± 0·161. The change in values for testosterone for the individual groups were: group 1 from 4·05 ± 0·677 to 2·672 ± 0·296 ($P < 0{\cdot}02$); group 2 from 2·280 ± 0·035 to 2·669 ± 0·343 ($P < 0{\cdot}03$); and group 3 from 1·98 ± 0·232 to 3·331 ± 0·335 ($P < 0{\cdot}05$). Similarly for DHT the changes were: group 1 from 1·794 ± 0·167 to 1·117 ± 0·173 ($P < 0{\cdot}02$); group 2 from 1·856 ± 0·431 to 1·39 ± 0·174 (NS); and group 3 from 3·331 ± 0·335 to 1·182 ± 0·154 ($P < 0{\cdot}05$).

Previous oral contraceptive therapy did not influence hormonal levels prior to treatment and the response to hormone therapy in each group.

The changes in total testosterone and DHT did not correlate with clinical improvement or fall in sebum excretion in individual patients.

These results suggest that the clinical response to cyproterone acetate is related to its peripheral action as an androgen antagonist rather than suppression of either the pituitary–adrenal or pituitary–ovarian axes.

Effect of 13-*cis*-retinoic acid and erythromycin on facial surface and follicular skin microflora in nodulo-cystic acne

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Fifty-one patients with nodulo-cystic acne have been randomly allocated to treatment groups of (1) 0·5 mg/kg bw 13-*cis*-retinoic acid (RA) daily (2) 1 g erythromycin daily (3) the combination. 13-*cis*-RA was prescribed for 16 weeks and erythromycin for 24 weeks.

At 0, 16, 24 and 32 weeks, facial surface skin microflora was sampled using the Williamson and Kligman technique. Facial follicular skin microflora were sampled using the cyano-acrylate gel technique (Holland *et al.*, *J. Appl. Bact.* 1974, 37, 289).

There was a significant reduction ($P < 0{\cdot}005$) in the surface anaerobic bacteria in all three treatment groups during the respective treatment periods, though more marked in 13-*cis*-RA groups (58% reduction in 13-*cis*-RA group, 36% in erythromycin group and 65% in the combination group). The follicular anaerobic bacteria showed a similar but greater pattern of reduction (67%, 51% and 73% respectively). The anaerobic bacteria returned to pretreatment numbers in the erythromycin alone group at 32 weeks but were still significantly reduced in the other two groups ($P < 0{\cdot}005$).

The surface aerobic bacteria were significantly reduced ($P < 0{\cdot}01$) in all treatment groups at 16 weeks, though to a lesser extent than the anaerobic bacteria, and had returned to pretreatment numbers at 32 weeks. There was no significant reduction in the follicular aerobic organisms during therapy except in the 13-*cis*-RA alone group at 16 weeks.

Erythromycin produced no change in the sebum excretion rate, whilst 13-*cis*-RA produced an 80% reduction. The acne had improved by 50% in the erythromycin group and 80% in the 13-*cis*-RA groups at 24 weeks. It is concluded that whilst erythromycin directly affects the bacterial population, 13-*cis*-RA affects their micro-environment, and that follicular aerobic bacteria are less dependent on sebaceous material.

Effect of 13-*cis*-retinoic acid on inflammatory responses in acne vulgaris

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Vitamin A, from which 13-*cis*-retinoic acid (RA) is derived, has been reported as an immunopotentiator and other synthetic retinoids are known to possess adjuvant properties. To determine whether any of the beneficial effects of 13-*cis*-RA in acne may be due to stimulation of the immune system, leukocyte subpopulations, C3 and immunoglobulin levels were measured in the peripheral blood of acne patients treated with 0·5 mg/kg 13-*cis*-RA for 4 months. Measurements were made prior to therapy and at 4 weekly intervals.

Results showed that the immune system of these patients had been stimulated at the humoral level at 8 weeks of therapy. Stimulation was evidenced by increased levels of IgG, IgA and IgM and also increased numbers of helper T cells. This stimulation of T cells was followed by significantly increased numbers of B cells with surface immunoglobulins at 16 weeks.

This evidence suggests that there is some initial stimulation of the immune system in acne patients treated with 13-*cis*-RA which may be a contributory factor to their marked clinical improvement.

Modulation of keratin expression in basal, spinous and granular epidermal cells

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Epidermal tonofilaments are composed of a complex group of keratins. These major differentiation products are synthesized by living keratinocytes and modified during the formation of mature corneocytes. More detailed study of keratins from different layers of living epidermis has revealed that alterations in keratin expression occur during keratinocyte maturation prior to cornification.

Basal, spinous and granular cell fractions of mouse and human epidermis were prepared by trypsin treatment and percoll gradient centrifugation. Keratins were isolated from cytoskeletal preparations of each fraction and from pure statum corneum. These were characterized by one-and two-dimensional (IEF and NEpHGE) electrophoresis and peptide mapping after digestion with V8 protease.

Basal cell fractions contained two major keratins (human 59K, 52K; mouse 59K, 54K), the larger of each pair belonging to the basic keratin group (pI 6·5–8·0) and the smaller to the acidic group (pI 5·0–5·5). Spinous cell fractions contained two additional major keratins (human 68K basic, 57K acidic; mouse 67K basic, 57K acidic) and in the granular cell fractions, these two additional keratins became the major components. In each case basic–acidic keratin pairs were present in a 1:1 ratio. Peptide mapping confirmed that the same-sized keratins from the different fractions were identical, all four keratins having unique maps. Corneocytes contained smaller and more acidic keratins (humans 64K, 57K, 55K; mouse 63K, 61K, 57K, 56K) and peptide mapping indicated that these were produced by proteolytic modification of the 67K/68K basic and 57K acidic keratins abundant in the granular layer.

We conclude that cells which attain a suprabasal position express two additional keratins which later undergo further modification producing mature corneocyte keratin. The 'basal-cell type' keratins appear to be quantitatively reduced as terminal differentiation progresses.

Stimulation of keratinocyte attachment and spreading by keratinocyte-derived matrix

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The aim of this work was to study the influence of extracellular matrix components on rat keratinocyte adhesion and growth. This was investigated *in vitro* using a low Ca^{2+} growth medium (MCDB 151) in which the cells remain as a monolayer. Cells were isolated with dispase and plated at $10^5/cm^2$ in 35-mm tissue culture petri dishes (controls) or dishes on to which a layer of 15 μg of purified matrix component (fibronectin, laminin, type IV collagen, type I collagen or laminin plus type IV collagen) had been deposited. Cultures were incubated at 37°C in an atmosphere of 5% CO_2 in air. Eighteen hours after plating, dishes were washed, the number of spread cells was determined by counting several fields under phase microscopy and the total number of attached (spread plus round) cells was then determined by trypsinization and counting. Growth was measured by counting the number of attached cells at intervals for up to a week. These experiments showed that none of the isolated matrix substrata tested had a substantial effect on the adhesion and subsequent growth of keratinocytes. This led us to reason that keratinocytes produced their own matrix for attachment and spreading irrespective of the substratum provided. To test this, extracellular matrix laid down by keratinocytes was prepared by removing the cells with 0·1% triton/5 mM NaOH. When freshly isolated keratinocytes were plated on to this keratinocyte-derived matrix, attachment and spreading occurred much more rapidly and after 18 h 2–3 times as many cells were attached as in controls. These observations confirm the importance of an organized cell-derived matrix for keratinocyte attachment and spreading and suggest the existence of hitherto unrecognized adhesion requirements for these cells.

Serum calcium levels and epidermopoiesis

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The growth and differentiation of both mammalian and human epidermal cells *in vitro* show a marked dependence on medium calcium concentrations. At low calcium levels (< 0·2 mM) cells divide rapidly and show a reduced tendency to differentiate. An increase in the concentration of calcium in the medium leads to inhibition of growth and induction of differentiation. The question as to whether this mechanism operates *in vivo* is of obvious importance. Although the levels of serum calcium are usually maintained within strict limits, fluctuations or local variations in calcium concentrations may occur. To assess the sensitivity of epidermal cells *in vivo* to serum calcium levels we have induced hypercalcaemia in rats and measured the effect on epidermal thymidine labelling index (LI) and mean epidermal thickness (MET). As intraperitoneal injection of $CaCl_2$ produced only transient hypercalcaemia, rats were maintained in a hypercalcaemic state by hourly injections for a period of 8 hours. After a further 16 h biopsies were taken and the LI and MET determined. Hypercalcaemia produced a marked decrease in LI (2.20 ± 1.26, n = 6) compared with saline-injected controls (7·57 ± 1.25, n = 6) paralleling the *in vitro* situation. The MET also showed a slight but not statistically significant decrease (19 1 ± 1·7 versus 21·3 ± 3·1 for controls). An attempt was also made to induce hypocalcaemic states by injections of calcitonin. Although a decrease of serum calcium was noted no increase in LI or MET was observed. These results suggest that whilst the calcium effect may operate under some circumstances *in vivo* it is not a major regulator of epidermopoiesis.

Isolation and characterization of the stratum compactum—a distinct region of the stratum corneum

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Evidence has been accumulating to suggest that there are morphological differences between cells of the upper (dysjunctum) and lower (lucidum) stratum corneum. In an attempt to understand what these differences are, we have isolated a specific region of the stratum corneum which we have termed the stratum compactum. This isolated region appears to be analogous to the stratum lucidum.

Light and electron microscopical evidence reveals that the isolated region is approximately five to seven cells thick. Interaction between the cells is considerable since mechanical agitation, solvent extraction and enzyme digestions fail to disperse the cells. Initial indications are that this region presents a barrier to the permeation of water.

Lipid analysis shows that its lipid profile is different from that of the underlying granular layer while its fatty acid composition differs to some extent from that of the more superficial stratum corneum cells. The analysis also suggests that the *o*-acyl glycolipid described by Gray, White & Majer (*Biochem. Biophys. Acta* 1978, **528,** 127) is not directly responsible for the barrier function of the stratum corneum.

The time course of the epidermal response to low dose X-irradiation

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The development of a non-invasive test for the detection of low dose irradiation to the skin has considerable practical importance. Previously we reported a dose-dependent decrease in epidermal labelling index and rate of desquamation when assessed 6 days after exposure to 90 keV X-rays (Barton *et al., Br. J. Derm.* 1983, **108,** 236). We now wish to report the time course of these and other changes. Consenting patients undergoing radiotherapy for neoplastic disease were exposed on randomized sites of the inner arm to a 100 rad dose of 90 keV X-rays. Biopsies were taken after 1, 3, 7 and 14 days and processed for thymidine autoradiography and non-specific esterase (NSE) activity. The rates of desquamation were assessed by a forced desquamation method.

The changes in mean labelling indices, NSE activity in the granular layer and of forced desquamation are shown in Table 1. The labelling index is significantly decreased at 1, 3, and 7 days but not at 14 days. Desquamation rates and NSE activity show a much higher individual variation but show exactly the same trend.

The results indicate that X-irradiation affects the epidermis at all levels and the effects seen are not simply due to a reduction in cell production since all occur with a similar time course. The changes in epidermal differentiation may be used to monitor the effects of irradiation although the techniques used

TABLE 1.

		Days post-irradiation			
	Control	1	3	7	14
Labelling index	6·38	2·65	0·95	1·16	3·80
Corneocytes released	105·84	53·96	50·36	67·02	104·88
NSE activity	66·00	41·19	62·96	104·48	87·42

show too great an individual variation to be of practical use. Quantitative dansyl choride fluorescence clearance is being explored now as this is less variable and is easily performed.

Lichen simplex chronicus and atopy

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It is frequently asserted that lichen simplex chronicus (LSC) is a manifestation of the atopic state but to our knowledge there is no published evidence as to the validity of this statement. In this study we have investigated the relationship. Altogether twenty-five patients with LSC were studied and compared for features of atopy with a similar number of age- and sex-matched control subjects. The atopic state was thought to be present if (a) they gave positive reactions to two or more allergens on intracutaneous testing or (b) gave a positive family history of atopy in a first degree relative and themselves had a serum IgE level greater than 50 i.u./l and/or exhibited white dermographism. Patients were accepted as having LSC if they had an irritant lichenified circumscribed scaling patch and the biopsy satisfied the published histological criteria.

Based upon these criteria we found two main groups of patients with lichen simplex chronicus.

(1) A group of older, mainly female patients who were not atopic. None of these patients had scalp or nuchal involvement. This group constituted 48% of patients studied.

(2) A group of patients, mainly male, exhibiting both LSC and the atopic trait. This group amounted to 28% of patients studied and included those patients with LSC from the Indian subcontinent.

The incidence of atopy in this study is greater than expected in the general population from published data, and in our group of age- and sex-matched controls.

Growth hormone status in psoriasis

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The humoral component in psoriasis has recently been identified as an elevation of serum growth hormone (Weber *et al.*, *Arch. Dermatol. Res.* 1981, **270**, 129), which was claimed to be the dominant (aetiological) factor in psoriasis. We have compared the growth hormone status of twelve patients with widespread psoriasis with five eczema patients and six normal subjects. Male subjects, average age 33 years, fasted overnight and remained in bed. A cannula was inserted into a forearm vein at 7.30 a.m. and 10 ml blood was collected every 30 minutes until noon. Plasma was prepared for radioimmunoassay of growth hormone (HGH) and prolactin. Results were expressed as mU/l.

The overall mean and peak HGH values of the three groups appeared strikingly different: mean HGH (± s.e.m.) in psoriasis 4·4 ± 1·4, eczema 2·7 ± 1·7, controls 1·2 ± 0·3; peak HGH in psoriasis 15·6 ± 6·5, eczema 7·7 ± 6·1, controls 4·4 ± 1·8. Yet the differences were not significant (Wilcoxon rank sum test) and can be traced to five psoriasis patients and one eczema patient with exceptional values (mean > 3 mU/l). The remaining seven psoriatics had a mean HGH of 0·9 ± 0·2, below that of controls, with a peak of only 1·7 ± 0·5. Of the five psoriatics with raised HGH (mean 9·3 ± 1·7; peak 35 ± 10; $P < 0{\cdot}01$ against controls), one had a prolactin peak exactly coincident with the HGH peak and was stressed by the experimental procedure. This subgroup of patients was distinguished by leanness, but not by age or the total area of skin affected.

We conclude that elevated plasma HGH cannot be the cause of psoriasis, as suggested by Weber *et al.*, since a majority of our patients had normal hormone levels, but in some psoriatics raised plasma HGH may be a secondary feature of their skin disease.

Methotrexate pharmacokinetics in psoriatics developing hepatic fibrosis

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Methotrexate (MTX) is a well-established form of treatment for severe resistant psoriasis. Unfortunately hepatic fibrosis has emerged as a long-term side-effect.

Work by Noble (*J. Inv. Derm.* 1975, **91**, 69) showed three patterns of MTX handling in psoriatics on oral MTX, one of these groups having a long half-life. The present study was devised to see if there were any differences in MTX handling in psoriatics who had and had not developed hepatic fibrosis.

Seven psoriatics were selected who had developed varying degrees of heptic fibrosis and each was matched with two controls for age, alcohol intake, route of administration, duration of therapy and total dose. MTX profiles were then obtained for 7 days following a standard dose, assays being performed by radioimmunoassay.

Taken as whole groups, there was no significant difference between the profiles of fibrotics and non-fibrotics. However, when analysed as a case–control study by multiple linear regression, half-life was significantly shorter in the fibrotic group $0{\cdot}05 > P > 0{\cdot}025$. One reason for this could be that in those patients developing fibrosis, tissue (i.e. liver) uptake is more avid. Alternatively, a damaged liver may not metabolize and excrete MTX as efficiently, reducing entero-hepatic circulation and resulting in more rapid renal excretion. To try to clarify this, future studies are planned to measure liver and tissue concentrations of MTX.

Inhibition of phospholipase A_2 activity from epidermis of non-lesional psoriatic skin by serum albumin

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Phospholipase A_2 activity from non-lesional psoriatic epidermis is significantly greater than that from normal epidermis (Forster *et al.*, *Br. J. Derm.* 1983, **108**, 103). Phospholipase A_2 activity in fluid from suction blisters is very low (Yardley *et al.*, *J. Invest. Dermatol.* 1983, **80**, 380); also the enzyme activity in the blister roof is less than in epidermis obtained by dermatome. This may be due to the presence of serum. We therefore studied the effects of serum, blister fluid and human serum albumin (sometimes included in the assay for phospholipase A_2; Badas & Hay, *Life Sci.* 1980, **26**, 1721) on phospholipase A_2 activity from non-lesional psoriatic epidermis.

Epidermal phospholipase A_2 activity was determined as previously described. Inclusion of serum and blister fluid in the assay mixture reduced the enzyme activity. Inclusion of human serum albumin in the assay mixture reduced the activity of the epidermal enzyme in a concentration-dependent manner: final concentrations of 3·3, 6·6 and 10·0 mg/ml reduced the enzyme activity by 26%, 50% and 55% respectively. This contrasts with the findings of Kupferberg *et al.* (*J. biol. Chem.* 1981, **256**, 6274), and our own observations, that human serum albumin stimulates snake venom phospholipase A_2 by relieving product inhibition.

We previously suggested that enhanced phospholipase A_2 activity in non-lesional psoriatic epidermis could lead to increased concentrations of eicosanoids and to a psoriatic lesion. Spontaneous clearing of psoriatic lesions and their improvement by serum injection (Stankler, *Br. J. Derm.* 1969, **81**, 203) is recognized. The reduction of phospholipase A_2 activity by the albumin component of the serum could explain these phenomena.

The effect of etretinate on cyclo-oxygenase and lipoxygenase products of arachidonic acid in psoriatic skin

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Etretinate, an aromatic retinoid, is an effective oral therapy for psoriasis although its mechanism of action is not known. Previous work has shown that involved psoriatic skin contains raised levels of arachidonic acid (AA), prostaglandin E_2 (PGE_2), 12-L-hydroxy-5,8,10,14-eicosatetraenoic acid (12-HETE) and leukotriene B_4 (LTB_4) compared with uninvolved skin. We have studied the effect of etretinate on AA metabolism in skin.

Eight psoriatic patients gave informed consent. Chamber fluid from abraded involved and uninvolved skin was collected before and at weekly intervals after commencing etretinate therapy (50 mg/day) for 6 weeks. The chamber fluid was analysed for AA, 12-HETE and PGE_2 by GC-MS and for LTB_4 by the microdroplet chemokinesis assay.

Scaling, infiltration and to a lesser extent erythema improved in seven patients but none cleared completely. One patient deteriorated during therapy. Before etretinate AA, 12-HETE but not PGE_2 levels were higher in chamber fluid from involved compared with uninvolved skin. Concentrations (ng/ml, mean ± s.e.) found for involved and uninvolved skin respectively were: AA, 210 ± 40 and 83 ± 18, $P=0{\cdot}018$; 12-HETE, 38·7 ± 5·7 and 13·6 ± 2·2, $P=0{\cdot}002$; PGE_2, 7·2 ± 1·6 and 12·4 ± 2·1, $P=0{\cdot}062$ (paired t-test).

There was a significant decline in the mean levels of AA, 12-HETE and PGE_2 in involved skin during etretinate therapy (regression analysis). Changes in uninvolved skin were not significant. LTB_4 was found in involved psoriatic skin before and during etretinate therapy. Etretinate did not appear to affect LTB_4 levels.

In conclusion, we have demonstrated that etretinate therapy reduces the increased levels of AA and 12-HETE in lesional skin of psoriasis. Concomitant clinical improvement in the severity of the disease was also observed.

The effects of topical application of leukotriene B_4

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The powerful chemo-attractant, leukotriene B_4 (LTB_4), has recently been identified in scale and chamber fluid from lesional psoriatic skin. We now report the effects of topical application of chemically synthesized LTB_4 to the skin of normal volunteers.

LTB_4 (0·5–500 ng) in ethanolic solution was applied to the skin, evaporated and occluded for 6 hours. As controls, 20-carboxy-LTB_4 (1 μg) and 8,15-dihydroxyeicosatetraenoic acid (1 μg), which are chemically similar to LTB_4 but have negligible chemotactic properties, and also ethanol alone, were similarly applied. Histological studies were done at intervals after application of 100 ng LTB_4.

No visible reactions were seen at control sites. Six subjects developed erythema and swelling (onset 12–24 hours) at sites of application of 5 ng LTB_4 or more, one subject reacting to 10 ng or more. Diameters of the reactions were dose-related. Biopsies showed dermal and epidermal neutrophil infiltrates, forming numerous discrete subcorneal micro-abscesses by 24 h. Biopsies from 48 h to 7 days showed epidermal changes consistent with a resolving inflammatory reaction, with loss of the granular layer and slight parakeratosis at 48 h.

The histological features and absent control reactions indicate that the LTB_4 reaction is not due to non-specific chemical effects. The reaction may represent a pharmacological and histological model of the computed for each wavelength. Although peak response occurred at 405 nm, measurable sensitivity extended down to 254 nm.

The conclusion from this study is that, provided development techniques are standardized, diazochrome KBL is a suitable chromophore for a UV film badge dosimeter.

Skin collagen content in sex-hormone treated and untreated post-menopausal women

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Oestrogen therapy is gaining widespread use as its effects on the relief of symptoms of the climacteric and its prevention of osteoporosis becomes better appreciated. Little work has been done on the effects of oestrogens on the skin and though McConkey (*Ann. Rheum. Dis.* 1967, **26,** 219) connected thin skin and osteoporosis, the association with the menopause has never been made. Shuster *et al.* (*Br. J. Derm.* 1975, **93,** 639) showed that skin thickness, closely associated with skin collagen content, showed a significant sharp decrease after the fifth decade, unlike in men.

We looked at the skin collagen content in post-menopausal women on hormone replacement therapy and compared this with untreated and age-matched population.

Skin biopsies were taken from twenty-nine post-menopausal women who had not been given hormone replacement therapy (HRT) and from another twenty-six women who had been treated with oestrogen and testosterone implants for 2–10 years. The collagen content of the skin was calculated from the hydroxyproline value and the result was expressed in $\mu g/mm^2$ in relation to the surface area of the skin. (Shuster *et al.*, *Br. Med. J.* 1970, **4,** 722; Neuman *et al.*, *J. Biol. Chem.* 1950, **186,** 549).

The mean hydroxyproline content and therefore the mean collagen content in the skin was found to be 48% greater in the treated than in the age-matched untreated women. This was statistically significant ($P < 0{\cdot}01$). The implication is that oestrogen and/or testosterone prevents the decrease in collagen that occurs with ageing and protects skin the same way as it protects bone in the post-menopausal women.

Epithelialization of transplantable fibrous dermal collagen

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In our investigation of the use of cell-free fibrous dermal collagen, prepared by trypsin-treatment of skin, as a replacement for lost or damaged skin, we have made an *in vivo* and *in vitro* study of epithelialization of pig dermal collagen.

Pig collagen heterografts, including collagen stabilized by aldehyde cross-linking, eventually became epithelialized when implanted into excised skin wounds in rats as occurs with rat collagen allografts (Oliver *et al.*, *Br. J. exp. Path.* 1977, **30,** 88). This process, involving migration, mitosis and differentiation of recipient epidermal cells, occurred slowly and took 4 weeks for grafts measuring 1 × 1 cm.

The acceptability of fibrous dermal collagen as a substrate for epithelial growth was further investigated *in vitro* using explants of new-born rat skin and by seeding the collagen with dispersed epidermal cells. While epithelial outgrowth from small (0·2 × 0 2 cm) skin explants declined after 8 days, outgrowth of a multi-layered epidermis continued for 12 days on pig collagen pre-seeded with rat fibroblasts. Dispersed epidermal cells, prepared from new-born rat skin, and seeded on pieces of pig collagen (1 × 1 cm) in 35-mm petri dishes (2×10^6 cells/dish) quickly settled to form a complete epithelial cover of 3–4 cell layers with desmosomal junctions. After 4 days incubation the collagen pieces were grafted into excised skin wounds in rats and biopsied after 6 days. Histological examination revealed complete epithelialization with differentiation of a stratum granulosum in some grafts and greater epithelial coverage in all grafts than would be expected by recipient epithelial migration alone at 6 days.

These results suggest the feasibility of developing a transplantable 'skin' by growing fibroblasts and epidermal cells on fibrous dermal collagen *in vitro* prior to grafting.

Assessment of a skin surface conductance and capacitance apparatus

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A new instrument for measuring skin hydration, based on epidermal electrical impedance at 3·5 MHz, has been assessed. The hydrometer is said to measure the resistive and capacitative components of skin and to eliminate the effect of lead inductance. The accuracy with which the instrument performs was ascertained by building circuits comprising various carbon resistors and ceramic capacitors and the values of the components used were determined by using a Q-meter and Wayne-Kerr bridge. Zero drift was identified but could be eliminated by periodic re-zeroing. The hydrometer was set up to give correct resistance and capacitance readings at the manufacturer's calibration point. The range over which indicated conductance values were accurate to any specific limit was dependent on the capacitance in the circuit. Indicated capacitance was not independent of the resistor in the circuit. A matrix of conductance and capacitance points was plotted from which an area was identified in which useful measurements could be made.

Readings were taken at ambient temperature 22–24°C and relative humidity 38–55%. There was minimal correlation ($\tau=0{\cdot}15$) between humidity and capacitance and conductance. A mean of three readings over a 5-min period was optimal for achieving consistent readings.

In twelve normal subjects (19–37 years) six different body sites had differing capacitance and conductance values, e.g. face 3·2 pF, 22·1 $\mu\Omega^{-1}$, upper arm 1·5 pF, 8 6 $\mu\Omega^{-1}$, but symmetrical sites were similar. Over a period of 3 weeks, day to day variation at each site was minimal for each individual (e.g. forearm 2·0 ± 0·6 pF, 8·0 ± 1·5 $\mu\Omega^{-1}$) but there was some variation between individuals (mean of twelve subjects: forearm 2·45 ± 0·81 pF, 14·45 ± 6·85 $\mu\Omega^{-1}$).

Capacitance and conductance of ten subjects aged 70–91 years (mean 80 years) with normal skin was found not to be significantly different from that of the younger age group (e.g. upper arm, mean of ten subjects 2·4 ± 0·82 pF, 7·6 ± 4.6 $\mu\Omega^{-1}$) as compared with the mean of twelve younger subjects (upper arm 1·54 ± 0·66 pF, 8·59 ± 3·0 $\mu\Omega^{-1}$).

Computer-aided recall of dermatopathology data and its inter-relationships

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The storage of histological data at present does not usually enable slides to be recalled easily. Furthermore it is impossible to obtain quantitative data on specific histopathological features or their inter-relationships from the manual file system usually employed. For these reasons much of the information contained in dermatopathology libraries is inaccessible.

In order to improve this situation we have employed a file management system based on the Ashton-Tate program dBASE II in an Intertec Superbrain I microcomputer. This system has sufficient storage capacity for at least 1000 slides on one floppy disc. It enables data to be entered easily and unambiguously. Furthermore recall is now possible by name, file number, diagnosis or by histopathological features or by any combination of these. This technique greatly facilitates patient review and the compilation of data for histopathological investigation. The organization of information for storage and the operation of the program will be discussed and a study using this computer-based system to learn the determinants for epidermal cell death will be presented.

POSTERS

Elevated free testosterone in the saliva of women with acne and its reduction on cyproterone acetate and ethinyl oestradiol

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Raised derived free testosterone concentrations for women with acne have been reported (Lawrence *et al.*, 1981, *Clinical Endocrinology* **15**, 87). Free testosterone can be measured directly from saliva (Smith *et al.*, 1979, *Fertil. Steril.* **3**, 513) and a sensitive direct RIA (C or V 11% at 67 pmol/l) was used on several saliva samples from twelve women with long duration, moderate to severe acne of mean age 26 years. The mean value of 97 pmol/l (45–140) free testosterone was significantly higher ($P < 0{\cdot}01$) than the mean value of 63 pmol/l (35–97) free testosterone in saliva from a group of fourteen control women matched for age, weight and time sampling on random days of the menstrual cycle. Plasma testosterone concentration 2·1 nmol/l and sex hormone binding globulin mean levels (SHBG) 40 nmol/l in the women with acne were significantly different from the control values of 1 7 and 59 nmol/l.

A reduction (33%) of free testosterone concentration in the saliva to within the normal range was noted in acne patients treated with cyproterone acetate (2 mg) and ethinyl oestradiol (50 μg). This reduction was due to increased SHBG concentration (three- to fourfold) since plasma testosterone levels were unchanged during the therapy. It is concluded that free testosterone in saliva is a useful marker of androgenicity in acne.

Trilostane inhibits human sebaceous gland 3β-hydroxysteroid dehydrogenase: a probe for the action of adrenal androgens on sebum excretion

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3β-Hydroxysteroid dehydrogenase (3β-HSD) of human skin is concentrated in sebaceous glands and converts dehydroepiandrosterone (DHA) into 4-androstene-3, 17-dione (AE), a precursor of testosterone and 5α-dihydrotestosterone. This may provide a mechanism for the control of sebum secretion by adrenal androgen. A test of this hypothesis would be to inhibit sebaceous gland 3β-HSD. For this we studied trilostane (4,5-epoxy-17β-hydroxy-5α-androstan-3-one-2-carbonitrile). 3β-HSD activity was measured by the NAD^+ dependent conversion of [7α-^{3}H]DHA into [^{3}H]AE at 37°C in a homogenate of human forehead skin, pH 7 4. With 2·0 mM NAD^+ the apparent Michaelis constant for conversion of DHA into AE was 20 nM. The reaction was competitively inhibited by trilostane (Ki = 1·6 nM). Trilostane (up to 1·0 μM) did not inhibit conversion of testosterone into 5α dihydrotestosterone or bind to the human skin fibroblast androgen receptor. Trilostane is therefore a potent inhibitor of sebaceous gland 3β-HSD without other androgenic or anti-androgenic properties. Trilostane cream (5% microcrystalline dispersion) was applied to the foreheads of five males and five females (ages 19–25), all free of acne. Forehead sebum excretion rate (SER) was measured by the gravimetric method before and after 4 weeks of treatment. There were no statistically significant changes in SER (mean ± s.e.m., μg/cm^2/min) in males (before 0·83 ± 0·09; after, 0·89 ± 0·13) or females (before, 0·67 ± 0·18; after, 1·12 ± 0·26), which does not favour a major role for 3β-HSD in the control of SER in normal adults.

A comparison of the effects of oestrogen and cyproterone acetate and oestrogen and norethisterone on the bacterial population of the faces of women with acne

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Fifty-one female patients with acne grade > 1·0 (mean age 23) were stratified and randomized to one of three therapeutic groups. All groups received ethinyl oestradiol 50 μg days 5–25 and either cyproterone acetate 50 mg days 5–14 (group 1), cyproterone acetate 2 mg days 5–25 (group 2) or norethisterone 1 mg days 5–25 (group 3) for six menstrual cycles.

Counts of the aerobic and anaerobic bacterial populations of the face (results expressed as log count per 4 cm^2) were made in each patient mid-cycle prior to treatment and at 2, 4 and 6 months. Facial grade assessment, macule, papule and pustule counts and sebum excretion rates (SER) were performed at the same times. The bacterial counts were assessed in relation to therapy and correlated with clinical response.

No significant fall in the aerobic population count was observed in groups 2 and 3, but a significant fall was noted in the group 1 bacterial count at 6 months compared with the baseline count (3·31 cf. 4·41, $P = < 0{\cdot}01$). Similarly no significant decrease in the anaerobic population of the skin was achieved by therapies 2 and 3, but in group 1 a significant reduction was again evident at 6 months compared with baseline (2.89 cf. 4·13, $P = < 0{\cdot}01$).

Between-treatment analysis revealed no significant difference until 4 months when groups 1 and 2 were both significantly lower than group 3 ($P = < 0{\cdot}05$). At 6 months the reduction in group 1 was significantly lower than that observed in group 2 ($P = < 0{\cdot}05$) and group 3 ($P = < 0{\cdot}01$). The results for anaerobic populations were similar. There was no correlation between total aerobic or anaerobic counts and clinical response in any treatment group. However the falls in counts in group 1 correlated with reduction in SER. These results indicate that only ethinyl oestradiol 50 μg and cyproterone acetate 50 mg significantly affected the bacterial population of the face and these changes may be related to the reduction in SER with this therapy.

Serum hormone levels in female patients with acne

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In order to clarify the hormonal environment in female patients with acne, hormone levels which included serum LH, FSH, cortisol, oestradiol, progesterone, conjugated and free dehydroepiandrosterone (c-DHA, f-DHA), androstenedione, testosterone (T) and dihydrotestosterone (DHT) were measured in forty-one female patients with acne (Kligman's class III–IV) and the levels were compared with those in twenty-nine sex- and age-matched normal controls. Hormone levels were measured by radioimmunoassay using specific antiserum. Samples were obtained on the 7th day of menstrual cycle (follicular phase) and on the 7th day after ovulation judged by basal body temperature (luteal phase). T and DHT levels in patients with acne were $0{\cdot}47 \pm 0{\cdot}13$, $0{\cdot}20 \pm 0{\cdot}07$ ng/ml in follicular phase, and $0{\cdot}61 \pm 0{\cdot}29$, $0{\cdot}25 \pm 0{\cdot}10$ ng/ml in luteal phase, respectively. These levels were significantly higher than those in controls in both phases ($P < 0{\cdot}001$). f-DHA levels were higher in patients only in the luteal phase. The other hormone levels showed no significant differences between patients and control females. Mean levels of serum androgen in patients tended to be higher in luteal phase than those in follicular phase.

These results demonstrating high serum T, DHT and f-DHA levels in both follicular and luteal phases in patients with acne suggest a close relationship between circulating androgen levels and occurrence of acne although the source of these steroids remains to be investigated.

suggested that β_2m is not expressed on cells of the lower follicle. Although our study shows that this is not entirely correct there is a marked difference in β_2m staining intensity between the upper permanent and lower transient parts of the follicle. It has been proposed that the absence of β_2m expression is an indication of malignant transformation. In the lower hair follicle loss of immunochemically demonstrable β_2m accompanies normal differentiation.

Detection of epidermal basement membrane (BM) zone antigens in human fetal skin

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The epidermal BM has an important role in epithelial–mesenchymal interactions and seems to influence such processes as epidermal growth, movement and adhesion. Its function in epidermal ontogeny would be better understood if there was more information about the synthesis or localization of BM components during early embryogenesis.

Using indirect immunofluorescence we studied the localization of various BM macromolecules including bullous pemphigoid antigen (BP), laminin (LN), types IV (TIV) and V (TV) collagen and fibronectin (FN) in the skin of human embryos and fetuses of graded menstrual ages calculated from foot or crown-rump lengths. To avoid possible regional differences, samples of limb or limb bud were used. The source and dilution of primary and secondary antisera were standardized for each experiment. The results are summarized in Table 1.

TABLE 1.

Menstrual age (days)	BP	LN	TIV	TV	FN
54–56	ND	+	+	ND	+ + +
60–66	+	+	+	+	ND
88–95	+	+ +	+ +	+	ND
112–122	+	+ +	+ +	+	+ +
Adult (control)	+ +	+ + +	+ + +	+ +	ND

ND = not done; + slight; + + mod; + + + strong fluorescence.

There was a continuous BM linear staining at all fetal ages. However, BP staining appeared granular, and interrupted in the younger samples. TV also localized below BM, whereas FN was seen throughout the dermis.

These data indicate that many BM components can be detected during early embryogenesis. Examination of younger embryos will be needed to discover whether these components first occur together or sequentially.

Arachidonic acid and its metabolites in allergic and irritant contact dermatitis

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Little is known about pharmacological events in contact dermatitis. In this study suction blister fluid was obtained from inflamed skin 24 h after application of allergen (nickel sulphate or potassium dichromate) or irritant (benzalkonium chloride), and from control skin in human volunteers. Acidic lipid extracts were assayed for arachidonic acid (AA), prostaglandin E_2 (PGE_2), 12-hydroxy-eicosatetraenoic acid (12-HETE) and 5-HETE by gas chromatography–mass spectrometry, and for leukotriene B_4 (LTB_4) by an agarose microdroplet chemokinesis assay.

Mean AA (4144 ± 410 test, 2476 ± 209 control), PGE_2 (27·2 ± 2·1 test, 15·8 ± 2·1 control) and LTB_4 (0·09 ± 0·03 test, 0·01 ± 0·01 control) levels were significantly elevated in samples from allergic contact dermatitis compared with controls (ng/ml, mean ± s.e.m., n = 10, $P < 0{\cdot}02$ for each pair), whereas 12-HETE (24·5 ± 5·5 test, 15·3 ± 1·3 control) and pooled 5-HETE (6·8 test, 5·4 control) levels were not significantly raised.

In subjects with irritant dermatitis there were statistically insignificant differences between the levels of AA (2590 ± 412 test, 2117 ± 380 control), PGE_2 (19·3 ± 3·3 test, 14·1 ± 4·5 control) and 12-HETE (24·1 ± 6·2 test, 23·8 ± 8·3 control) in blister fluid from inflamed and control sites (ng/ml, mean ± s.e.m., n = 5, $P > 0{\cdot}05$ for each pair). LTB_4 levels were either undetectable or not significantly raised in blister fluid from inflamed sites.

Arachidonate cyclo-oxygenase and lipoxygenase pathways are therefore activiated in human allergic contact dermatitis, and may play a role in mediating some of the inflammatory events seen. Our results suggest that these pathways are less important in the irritant dermatitis reaction.

Personnel solar UVR exposure: a method of increasing the reliability of measurements made with film badge dosimeters

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Film badge dosimetry for UVR was originally described by Davis *et al.* (*Nature* 1976, **261**, 169) who used polysulphone film to measure UV-B (290–315 nm) radiation. This film, mounted in a 30 × 30 mm transparency holder (aperture 16 × 12 mm), has been used to monitor the solar UV-B exposure of normal subjects and patients receiving treatment for photodermatoses. The reliability of individual badge measurements after exposure to solar-simulated radiation (SSR: UV-B dose 40 mJ/cm^2) was calculated using a two-way ANOVAR. Data from eighty-seven irradiations showed a reliability of 0·65. Reliability increased when larger numbers of badges were used per exposure. Thus with two, three, four and five badges per exposure, reliability of the mean measurements was 0·79, 0·85, 0·88 and 0·90 respectively. A new badge mount has been designed with four circular apertures (6 mm diameter) with the same overall dimensions as the 'original' badge. Eighty irradiations were monitored by individual badges of this type. The mean of the four measurements from each badge had a reliability of 0·83. This is a 30% increase in reliability compared with the 'original' badges. Although a smaller area of film is used for each reading, the mean UV-B doses (± s.d.) recorded by the new (39·4 ± 7·6 mJ/cm^2) and 'original' (39·6 ± 8·8 mJ/cm^2) badges were the same. The new badges are equally cosmetically acceptable to participants in studies and are more robust in structure than the 'original' badges, thus offering better protection for the fragile polysulphone film.

An assessment of the value of the vasoconstrictor assay in predicting differences in clinical efficacy between corticosteroid ointments

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The occluded vasoconstrictor assay is a commonly used guide to the likely topical potency of

Like his predecessors in the department, Jamieson, Walker and Low, Percival studied at continental clinics. During 1928 and 1929 he worked in Paris, Lausanne and Zürich with several leading dermatologists including Civatte, Dupont, Sabouraud and Bloch. In many ways his initial meeting with Bloch epitomised his character and style. Having spent a week at the Zurich clinic with minimal attention from the great man, he went to Bloch's laboratory and in no uncertain terms, told him that he had not travelled so far to join the end of his entourage. Bloch, although surprised and hurt, must have been impressed by this keen but forthright visitor, because he spend much time with him during the rest of his stay. Indeed, after this, they corresponded regularly not only about the capricious dopa reaction but about dermatological research in general.

It is hardly surprising that Percival soon saw beyond the 'see and treat' routine that was so much of dermatology in the late 1920s and 1930s. His intelligence, experience of experimental work, and ambition demanded a more inquisitive approach. He embarked upon pharmacological research and wrote a series of papers with C.P.Stewart on parathyroid hormone, calcium metabolism and the skin. He studied blood vessels in inflammatory skin disorders and was spurred on by the recent discovery of histamine to investigate the nature of inflammatory mediators in the skin. A paper with C.M.Scott on 'The action of blister fluid on the isolated rat uterus', published in 1931 indicated that mediators other than histamine were involved in some blistering skin diseases. This view, received very coolly by Sir Thomas Lewis, led to a short and strained meeting between Lewis and Percival. Not deterred, the young and enthusiastic Scot returned to his home base to continue his research, and Bloch described him as the *Feu Sacré* of dermatological research in Britain at that time.

Percival said that when he joined the department of dermatology in Edinburgh there was not a single histological specimen to be found in it and that biopsies were special occasions. Convinced of the diagnostic and educational value of histopathology, he was one of the key figures in Britain in establishing the diagnostic role of histopathology of the skin. He threw his full energy and enthusiasm into assembling a systematic collection of histopathological material and encouraged those who trained in the department to make full use of it. *Histopathology of the Skin* written with A.Murray Drennan and T.C.Dodds (1947) was well received and provided much practical help for pathologists struggling with dermatological sections.

In 1945 Sir Robert Grant, largely as a result of his association with Percival, donated £70,000 to the University and £10,000 to the Royal Infirmary of Edinburgh to enable a Chair of Dermatology to be established. This was the first University Chair of Dermatology in Britain and Percival was appointed its first professor. Recognition of dermatology as a full academic subject in its own right marked a major step in British dermatology. Under his guidance the main work of the department, apart from clinical trials, concerned the histopathogenesis of eczema, bullous eruptions and the reticuloses and the pathogenesis of acne. He was a most effective teacher and always pointed out to undergraduates that, in the examination of patients with skin disease, they had a unique opportunity to observe pathological processes running their course and to correlate cellular pathology and derangement of function with the clinical findings.

The main characteristic of the man was his intelligence; and that allied to charm of manner and a relaxed attitude to events (which enabled him to stand aside from them, to look at what was going on, and then intervene decisively when he judged the time was right) was the mainspring of his success. Most of his contemporaries in dermatology were either unaware of his great ability, or were vaguely disturbed by it. At times he seemed contemptuous of them, his main criticisms being that they were obsessed by morphological minutiae yet unconcerned about, and ignorant of, pathogenetic mechanisms. He also thought that London had much to learn from Edinburgh on dressing regimens and the out-patient treatment of skin diseases. He held strong views, and there was an emotional barrier to accepting facts that ran counter to his own position. He was formidable in dispute but if a case was presented objectively, logically, and firmly, he could eventually be persuaded to change his stance. However, he ran circles around those who were ill-prepared and did not know their stuff.

In many ways Professor Percival was a master of serendipity, using his natural ability to cope with or exploit the next situation which presented itself. Failure to build on the many foundations which he established seemed to be a personal characteristic, if not a flaw. This makes him sound like a butterfly flitting from flower to flower, but if he was, then he belonged to an unusual species, and visited many exotic gardens. Once committed to a project he dropped everything else and threw all his own energy, and much of others, into seeing it through. He was so thorough that having completed the work, he seldom returned to extend or develop it, as this appeared to bore him. He was a genuine initiator rather than imitator and left much for others to develop.

Professor Percival thought that life was fun. He held strong views, not always in the direction of the prevailing wind, and never lost his forthright manner. He enjoyed challenges, ignored or stood aside from bureaucratic bungling, gave staunch and lifelong support to those who worked with him, and established an academic department with a genuine *esprit de corps*. Those who knew him realized that he could do almost anything that he set his mind to. At one time his knowledge of jade was unrivalled in Britain and he always knew a lot about antiques. By all accounts he was shrewd in his dealings on the stock exchange. The magnificient show of heaths, azaleas and rhododendrons at 'Woodcroft' are a tribute to his expertise and imaginative landscaping. He was an inveterate salmon fisher. To the end his intellect remained razor sharp and his enthusiasm never left him. Six months before he died he completed a remarkable 400 page text on 'Some aspects of the development of dermatology with special reference to the contribution of the Edinburgh Medical School' which will remain a monument to his erudition as well as to his love of the speciality and the University of Edinburgh. He was, without doubt, an outstanding man, and a luminary in British dermatology.

J.A.A.HUNTER

The aim of Martindale remains, as it always was, to provide information on the properties, action and uses of drugs. On the first two counts the 28th edition scores heavily. On the last, these reviewers are somewhat less sure that it succeeds. Whilst acknowledging that this is not intended to be a textbook of therapy there does seem to be some room for improvement. The determination to be unbiased appears to dominate, and where there is controversy about a drug's usage the aim has been to balance references for and against. In fact this seems to weaken the work. For many drugs there is no clear guidance on the therapeutic efficacy versus the scale of any side-effects.

When Martindale embarked on this extraordinary venture 100 years ago he sought the help, the flyleaf tells us, of Dr W.Wynn Westcott, who provided the clinical background and notes on the usage of the drugs then listed. The present editorial staff of twelve includes no medically qualified members and as a constructive suggestion for the future it might be well worthwhile strengthening this aspect of the book, perhaps by strengthening the team to include some practising clinical pharmacologists.

These criticisms apart there is no doubt that the 28th Edition of Martindale is packed with useful and, as far as we can tell, accurate information. It deserves to sell well and no doubt will.

S.O.B.ROBERTS
R.J.PYE

Symposium on Vascular Malformations and Melanotic Lesions. Edited by H.BRUCE WILLIAMS (1983) St Louis: C.V. Mosby. Pp. 421. Price £62.75.

This handsomely bound book describes the proceedings of a symposium sponsored by the Educational Foundation of the American Society of Plastic and Reconstructive Surgeons held in Montreal in 1980. At first sight vascular malformations and melanotic lesions might seem unlikely bed-fellows, but the treatment of such conditions not only occupies much time but also tests the ingenuity of many plastic surgeons.

The book might be a good *aide-mémoire* for those who attended the Symposium, but it would surprise me if many others will find it helpful as a reference source. With few exceptions most chapters seemed to summarize the subject in the form in which it was presented at the meeting. As a result there are too many repetitive pictures (e.g. countless series of photographs showing spontaneous resolution of strawberry haemangiomas) and the text is often too brief to give more than a superficial overview of the topic. Curious exceptions include the more comprehensive account of the leukocyte adherence inhibition test as a monitor in melanoma therapy which, in spite of its length, failed to convince me of its practical worth. The chapter on biological markers of human tumours details a number of hormones and tumour antigens which might help to make the diagnosis in tumours other than malignant melanoma, but does not mention specific metabolites produced by melanoma cells such as 5-S-cysteinyldopa.

I enjoyed the sections on haemangiomas and lymphangiomas but, bearing in mind that the book is intended to be educational, I would have thought that treatment policies for the various types of haemangiomas and their merits could have been detailed more concisely.

It must be a nightmare editing this sort of book but a stronger editorial policy could have made the classifications more uniform, standardized the nomenclature and removed many black and white photographs. From the comments in the discussions (summarized throughout the book) it appears that the meeting was lively and helpful, but this book provides little to contest the view that proceedings of such meetings are best left unrecorded. One would not have to look hard to find most of the information presented in this book covered elsewhere in a more concise and critical form.

J.A.A.HUNTER

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News and Notices

64th Annual Meeting of the British Association of Dermatologists, Belfast, 1984

The next annual meeting of the B.A.D. will be held at Queen's University, Belfast, under the presidency of Dr Martin Beare, on 27–30 June 1984. Those wishing to present a paper at this meeting should submit an Abstract (six copies) of not more than 200 words, to Dr G.Levene, Honorary Secretary, B.A.D., 7 John Street, London, WC1N 2ES, before 14 February 1984. The President's reception will be in Stormont Castle, and the Annual Dinner will be held at the Belfast City Hall, following a reception by the Lord Mayor. The ladies' programme will include visits to Hillsborough Castle, Mount Stewart House, Castleward House and the Ulster Folk Museum. The Contact Dermatitis Group of the B.A.D. will have a meeting on 26 June and the morning of 27 June, and the Surgical Group of the B.A.D. will meet on the morning of 27 June.

Paediatric Dermatology Course, Liverpool, May 1984

A 2-day course in paediatric dermatology will be held on 10–11 May 1984, at the University of Liverpool. The course is aimed at Senior Registrars and recently appointed Consultants in Dermatology, and will be limited to nine participants. Further details from Dr J.L.Verbov, c/o Postgraduate Dean's Office, Faculty of Medicine, University of Liverpool, P.O. Box 147, Liverpool L69 3BX. (Telephone 051 709–3114.)

Winter Skin Seminar, Aspen, Colorado, U.S.A., February 1984

The Tenth Annual Winter Skin Seminar will be held at the Given Institute of Pathobiology, Aspen, Colorado, on 12–17 February 1984. For further details contact: The Office of Postgraduate Medical Education, The University of Colorado School of Medicine, 4200 East Ninth Avenue, Box C-295, Denver, CO 80262. (Telephone 303 394–5241.)

British Dermatological Surgery Group

A British Dermatological Surgery Group has recently been formed and held an inaugural meeting in June 1983 in association with the annual meeting of the B.A.D. The B.D.S.G. is affiliated to the B.A.D. as a Special Interest Group. Membership is available to anyone interested in Dermatological Surgery. For further details contact Dr M.G.C.Dahl, Department of Dermatology, Royal Victoria Infirmary, Newcastle upon Tyne, NE1 4LP.

Dermatological Surgery Workshop, Newcastle upon Tyne, April 1984

A 3-day Workshop on Dermatological Surgery will be held in Newcastle upon Tyne on 12 to 14 April 1984. This is the first such workshop to be held in the United Kingdom and will be sponsored jointly by the British Dermatological Surgery Group and by the International Society for Dermatologic Surgery. It will be similar to the popular and successful workshops organized by the I.S.D.S. in the United States and elsewhere. It is intended for dermatologists in training and consultants who are keen to improve their skills in dermatological surgery. There will be practical sessions with pigs' feet and in cryosurgery, and the panel

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Abbreviations and units. Full names with uncommon abbreviations must be given with the first mention: new abbreviations should be coined only for unwieldy names and should not be used at all unless the names occur frequently. In the title unusual abbreviations should be identified, in the introduction and discussion they should be used sparingly. Abbreviations of units should conform with those shown below

gram(s)	g	minute(s)	min	molar	mol/l
kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3}g)	mg	second(s)	s	gravitational acceleration	***g***
microgram(s) (10^{-6}g)	μg	cubic millimetre(s)	mm^3	micrometre(s)	μm
nanogram(s) (10^{-9}g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12}g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^{131}I
hours(s)	h	milliequivalent	mmol		

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